LAB NOTEBOOK

by iGEM Thessaloniki



ARISTOTLE UNIVERSITY OF THESSALONIKI

aeth3r

iGEM Thessaloniki

Lab Activity Log - Rules & Usage Guidelines

Logging Policy for Experimental Days

1. Purpose

The **Lab Activity Log** is a centralized record of all laboratory work conducted by team members.
Its purpose is to ensure:

- Traceability of experiments
- Reproducibility of results
- Clear communication across wet lab members
- Continuity of work between lab sessions

All lab activities must be recorded **in real time or immediately after completion**.

2. Mandatory Logging Policy

- Every team member must **log each lab session** they participate in.
- Entries must be **complete, accurate, and concise**.
- No experiment or task should remain undocumented.
- Logs should reflect **what was actually done**, not what was planned.

3. Required Fields (Must Be Completed)

Each entry must include the following:

- **Date** – Day of the lab session
- **Time (Start–End)** – Duration of work
- **Name / Initials** – Person responsible
- **Project / Subproject** – Relevant workstream
- **Experiment / Task Title** – Short, descriptive name
- **Objective** – Clear purpose (1 sentence)

4. Experimental Documentation

To ensure reproducibility, include:

- **Materials / Reagents Used**
- **Method / Protocol Reference**
  (e.g., protocol version, literature source)
- **Sample / Cell Line**
  (e.g., A-549, MEC-1)

5. Observations & Results

- Observations / Notes
    - Record raw observations during the experiment
    - Include deviations, unexpected behaviors, or anomalies
- Result Summary
    - Brief outcome (no detailed analysis required)

6. Issues & Troubleshooting

- Problems / Deviations
    - Clearly state any errors, failures, or protocol changes
- Next Steps
    - Define what should happen next (repeat, optimize, analyze, etc.)
- Status
    - Must be selected from predefined categories:
        - Completed
        - Ongoing
        - Failed
        - Needs Repeat

7. Data & Traceability

- Data Link / Storage Location
    - Provide access to raw data (e.g., Google Drive)
- All results must be traceable to stored data

10. Accountability

- Each team member is responsible for their own entries
- Logs may be reviewed by supervisors or subteam leads
- Incomplete or missing entries compromise **data reliability and team workflow**

WET LAB LOG
iGEM Thessaloniki 2026

MAY 4TH 2026

Overview

- **Date** – Monday, May 4th 2026
- **Time (Start–End)** – 9:20 - 11:10
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Erriketti Moumka, Alexandros Vlachos
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Thawing of A-549 cell line
- **Objective** – The purpose of this day's experiment was to thaw the A-549 cell line to start the cell culture process.

Experimental documentation

- **Materials / Reagents Used**
  - ➢ T-25 flask (x1)
  - ➢ stripettes 5 mL (x4)
  - ➢ DMEM FBS 10% 1% p/s (16 mL)
  - ➢ 15 ml Falcon (x1)
  - ➢ 30 ml Falcon (x1)
- **Method / Protocol Reference**
  - ➢ In-house protocol for cell thawing
- **Sample / Cell Line**
  - ➢ cell line: A-549

Observations & Results

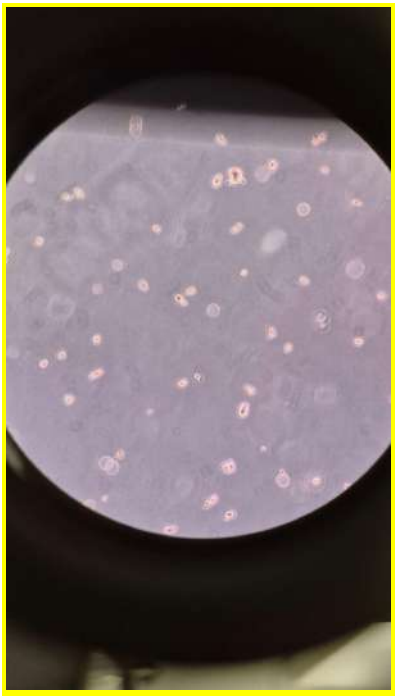
- **Observations / Notes**
  - The pellet of cells after the centrifuge step was present, indicating that the thawing step was done correctly.
  - After resuspending the pellet, the flask containing cells was observed, and no abnormalities were noted.
- **Result Summary**
  - The cell thawing process took around 20 minutes in total.
  - The cells were observed, and no abnormalities were noted.

Pictures: **link to folder**

Cryovial that was used: (yellow box - cryovial with orange cap 1/2)



T-25 flask after thawing



MAY 5TH 2026

Overview

- **Date** – Tuesday, May 5th 2026
- **Time (Start–End)** – 13:00 - 14:30
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Erriketti Moumka, Alexandros Vlachos
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Trypsin aliquots, medium change for cells, pipetting with gun-man training.
- **Objective** – The purpose of today's experiment was to prepare aliquots of Trypsin to store in the lab freezer for upcoming experiments (stock). Also, the T-25 flask was observed, and the medium was changed. Lastly, Erriketti Moumka and Alexandros Vlachos practiced their pipetting with the gun-man.

Experimental documentation

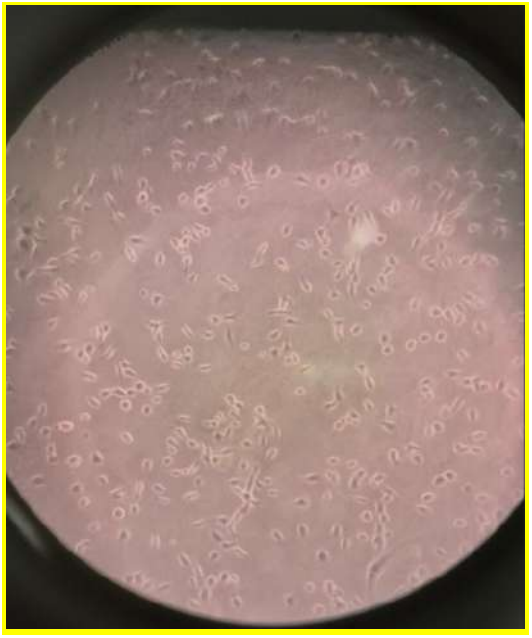
- **Materials / Reagents Used**
  - ➢ stripettes 5 mL (x5)
  - ➢ DMEM FBS 10% 1% p/s (4,5 ml)
  - ➢ 15 ml Falcon (x15)
  - ➢ 30 ml Falcon (x1)
- **Method / Protocol Reference**
  - ➢ In-house protocol for changing the medium
- **Sample / Cell Line**
  - ➢ cell line: A-549

Observations & Results

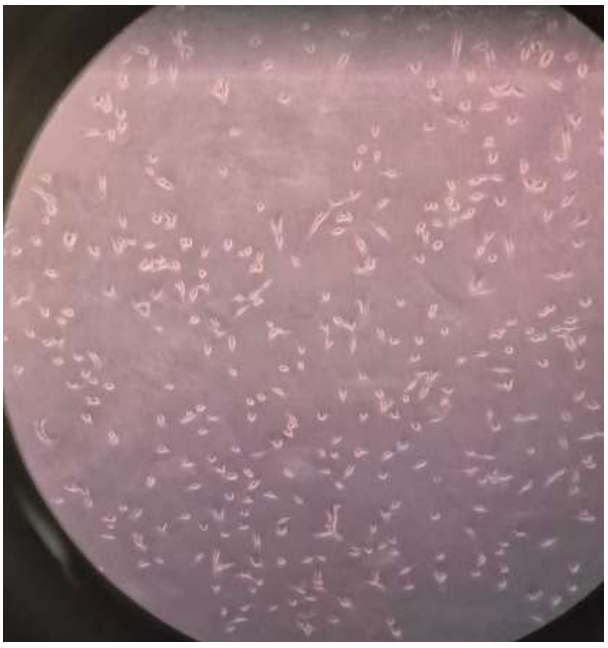
- **Observations / Notes**
  - The color of the medium had changed from a magenta color to a paler pink-orange color, indicating that the cells had started to metabolise and the pH of their environment was changing.
  - The surface the cells had taken up was around 40-45%.
- **Result Summary**
  - The cell thawing process, from the previous day, was done correctly.
  - The cells were observed, and no abnormalities were noted.

Pictures in drive: link

Day 1 - before medium change



After medium change



MAY 6TH 2026

Overview

- **Date** – Wednesday, May 6th 2026
- **Time (Start–End)** – 13:00 - 14:10
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Taxiarxis Kosmas
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Cell culture medium preparation, medium change for cells
- **Objective** – The purpose of today's experiment was to prepare cell culture medium for the A-549 cell line. Also, the T-25 flask was observed, and the medium was changed.

Experimental documentation

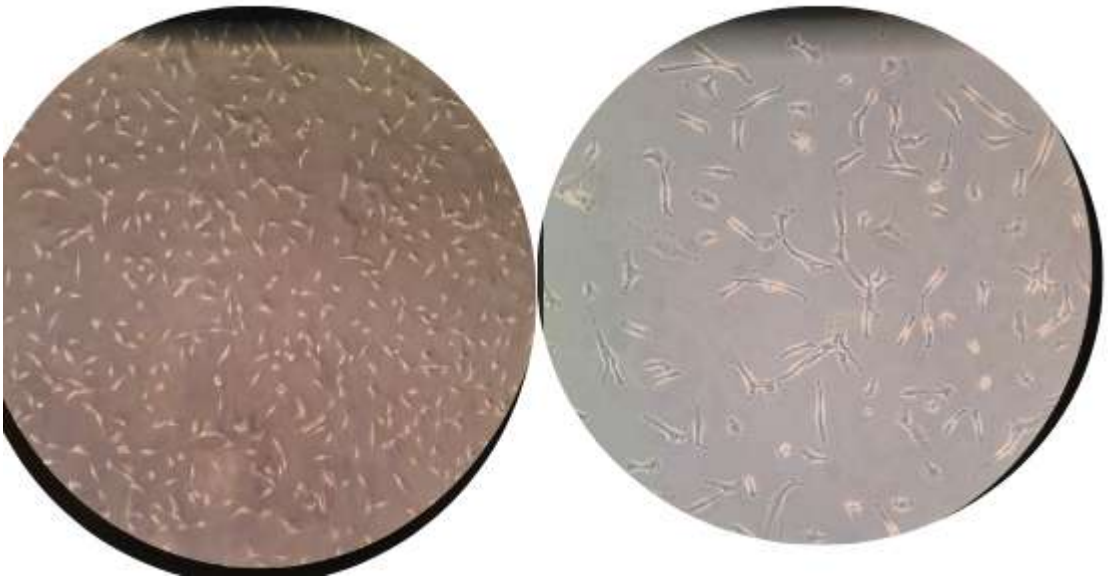
- **Materials / Reagents Used**
  - ➢ stripettes 5 mL (x4)
  - ➢ stripettes 10 mL (x1)
  - ➢ DMEM FBS 10% 1% p/s (4,5 mL)
  - ➢ 30 ml Falcon (x1)
  - ➢ 50 ml Falcon (x2)
  - ➢ Sterile syringe 10 mL (x1)
  - ➢ Sterile filter for syringe (x1)
  - ➢ Pen/strep (0,5 mL)
  - ➢ FBS (5 mL)
  - ➢ DMEM (44,5 mL)
- **Method / Protocol Reference**
  - ➢ In-house protocol for changing the medium
  - ➢ In-house protocol for cell culture medium preparation
- **Sample / Cell Line**
  - ➢ cell line: A-549

Observations & Results

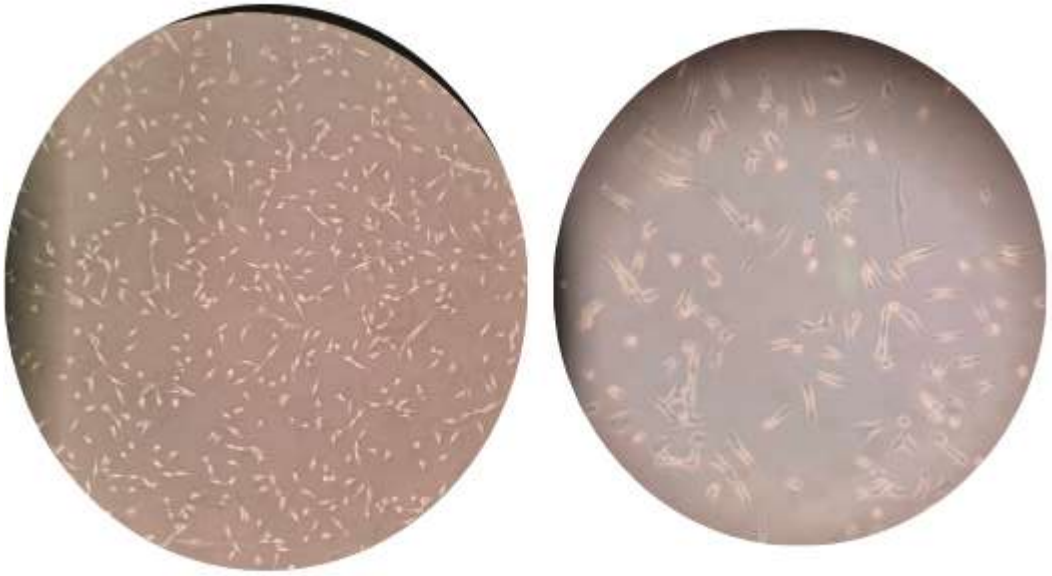
- **Observations / Notes**
  - The color of the medium had changed from a magenta color to a more pale pink-orange color, indicating that the cells had started to metabolise and the pH of their environment was changing.
  - The surface the cells had taken up was around 45%.
- **Result Summary**
  - Fresh cell culture medium was prepared.
  - The cells were observed, and no abnormalities were noted.

Pictures in drive: link

Before medium change:



After medium change



MAY 7TH 2026

Overview

- **Date** – Thursday, May 6th 2026
- **Time (Start–End)** – 14:20 - 16:00
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Taxiarxis Kosmas, Erriketti Moumka
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Checking of cell culture media preparation from the previous day, medium change for cells, pipetting with gun-man training.
- **Objective** – The purpose of today's experiment was to check the prepared cell culture medium for the A-549 cell line. Also, the T-25 flask was observed, and the medium was changed. Lastly, Erriketti Moumka and Taxiarxis Kosmas practiced their pipetting with the gun-man.

Experimental documentation

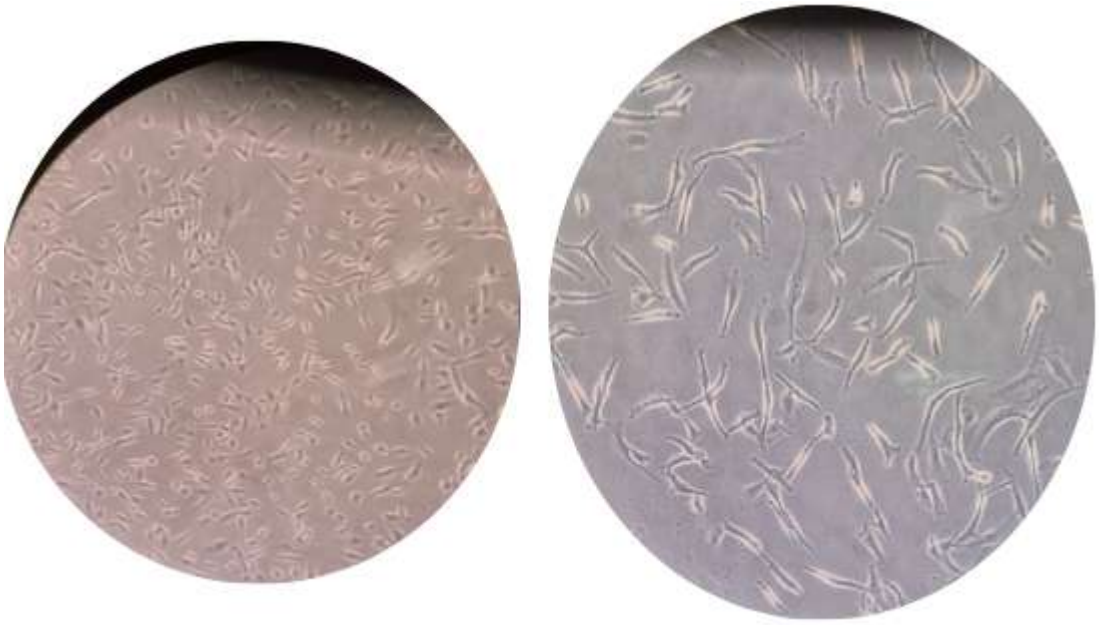
- **Materials / Reagents Used**
  - ➢ stripettes 5 mL (x4)
  - ➢ DMEM FBS 10% 1% p/s (4,5 mL)
  - ➢ 50 ml Falcon (x1)
  - ➢ Trypan Blue (10 µL)
  - ➢ 10 µL tips (x2)
- **Method / Protocol Reference**
  - ➢ In-house protocol for changing the medium
- **Sample / Cell Line**
  - ➢ cell line: A-549

Observations & Results

- **Observations / Notes**
  - The color of the medium had changed from magenta to a paler pink-orange, indicating that the cells had started to metabolise and that the pH of their environment was changing.
  - The surface the cells had taken up was around 50%.
  - A small aliquot of the cell culture medium prepared the previous day was examined for potential contamination using Trypan Blue staining and observation in a Neubauer chamber. No abnormalities or signs of contamination were observed.
- **Result Summary**
  - The cells were observed, and no abnormalities were noted.
  - The cell culture medium prepared on 06/05 was observed, and no abnormalities were noted.

Pictures in drive: link

Before medium change



After medium change



MAY 8TH 2026

Overview

- **Date** – Friday, May 6th 2026
- **Time (Start–End)** – 14:35 - 15:40
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Melita Lampropoulou, Erriketti Moumka
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Medium change for cells, pipetting with gun-man training.
- **Objective** – The purpose of today's experiment was to check the T-25 flask and change the medium. Lastly, Erriketti Moumka and Melita Lampropoulou practiced their pipetting with the gun-man.

Experimental documentation

- **Materials / Reagents Used**
  - ➢ stripettes 5 mL (x3)
  - ➢ DMEM FBS 10% 1% p/s (4,5 mL)
  - ➢ 50 ml Falcon (x1)
- **Method / Protocol Reference**
  - ➢ In-house protocol for changing the medium
- **Sample / Cell Line**
  - ➢ cell line: A-549

Observations & Results

- **Observations / Notes**
  - The color of the medium had changed from magenta to a paler pink-orange, indicating that the cells had started to metabolise and that the pH of their environment was changing.
  - The surface the cells had taken up was around 60%.
- **Result Summary**
  - The cells were observed, and no abnormalities were noted.

Pictures in drive: link

Before medium change



After medium change



MAY 9TH 2026

Overview

- **Date** – Saturday, May 9th 2026
- **Time (Start–End)** – 14:50 - 16:10
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Taxiarchis Kosmas, Erriketti Moumka
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Medium change for cells, cryopreserving cells in cryovials
- **Objective** – The purpose of today's experiment was to examine the T-25 flask and assess whether cell confluency was sufficient to proceed with the cryopreservation procedure.
    - ❖ ⅓ of the total flask volume was used for the reculture process.
    - ❖ ⅔ of the total flask volume was used for the making of 2 cryovials.

Experimental documentation

- **Materials / Reagents Used**
    - ➢ stripettes 5 mL (x12)
    - ➢ DMEM FBS 10% 1% p/s (14 mL)
    - ➢ 50 ml Falcon (x1)
    - ➢ 15 ml Falcon (x3)
    - ➢ PBS (3 mL)
    - ➢ Trypsin - EDTA 0,05% (3 mL)
    - ➢ Freezing Medium (2 mL)
    - ➢ 1.5 mL Eppendorf tube (x1)
    - ➢ Trypan Blue (10 μL)
    - ➢ 10 μL tips (x2)
    - ➢ 1.5 mL cryovials [white cap] (x2)
- **Method / Protocol Reference**
    - ➢ In-house protocol for changing the medium
    - ➢ In-house protocol for cryopreserving cells.
- **Sample / Cell Line**
    - ➢ cell line: A-549

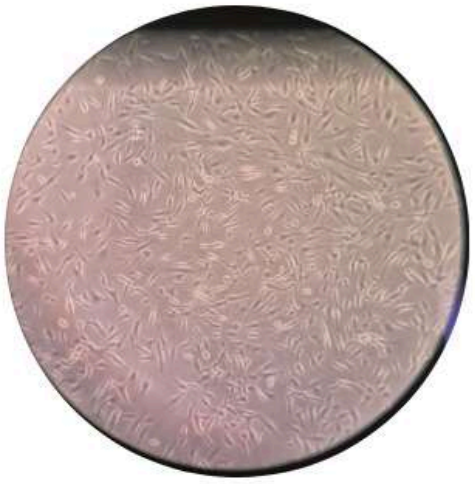
Observations & Results

- **Observations / Notes**
    - The color of the medium had changed from magenta to a paler pink-orange, indicating that the cells had started to metabolise and that the pH of their environment was changing.
    - The surface the cells had taken up was around 80%.
    - After the centrifugation, pellets were present in all 3 Falcon tubes.
    - Both cryovials were labelled (A-549, 9/5/26, MP-igem) in more than one place and are now stored in the yellow box (-80 °C) at the molecular laboratory.
- **Result Summary**

- The cells were observed, and no abnormalities were noted.
- The cryopreservation procedure was executed smoothly, within the safe-for-the-cells time slot.

Cell viability Excel: link
Pictures in Drive: link

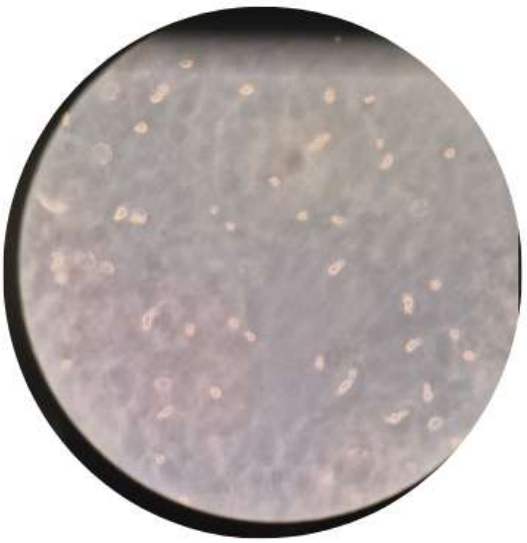
Before cryovials + reculture



BEFORE CHANGE + CRYOVIALS
(this is the density we saw before easter as well)

⅓ of the volume was recultures, the rest ⅔ were used for the 2 cryovials

After reculture



MAY 11TH 2026

Overview

- **Date** – Monday, May 11th 2026
- **Time (Start–End)** – 8:15 - 8:45
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Erriketti Moumka
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Medium change for cells.
- **Objective** – The purpose of today's experiment was to check the T-25 flask and change the medium.

Experimental documentation

- **Materials / Reagents Used**
  - ➢ stripettes 5 mL (x2)
  - ➢ DMEM FBS 10% 1% p/s (4,5 mL)
  - ➢ 30 ml Falcon (x1)
- **Method / Protocol Reference**
  - ➢ In-house protocol for changing the medium
- **Sample / Cell Line**
  - ➢ cell line: A-549

Observations & Results

- **Observations / Notes**
  - The color of the medium had changed from magenta to a paler pink-orange, indicating that the cells had started to metabolise and that the pH of their environment was changing.
  - The surface the cells had taken up was around 35%- 40%.
- **Result Summary**
  - The cells were observed, and no abnormalities were noted.

Pictures in Drive: link

Before medium change



After medium change



MAY 12TH 2026

Overview

- **Date** – Tuesday, May 12th 2026
- **Time (Start–End)** – 9:20 - 10:00 / 15:15 - 16:10
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Alexandros Vlachos (1st session) / Melita Lampropoulou (2nd session)
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Observation of cells to find their exact doubling time.
- **Objective** – The purpose of today's experiment was to check the T-25 flask and find the doubling time of the cells. Also, Melita Lampropoulou practiced her pipetting with the gun-man and flask handling.

Experimental documentation

- **Materials / Reagents Used**
  - ➢ stripettes 5 mL (x1)
- **Method / Protocol Reference**
  - ➢ -
- **Sample / Cell Line**
  - ➢ cell line: A-549

Observations & Results

- **Observations / Notes**
  - The color of the medium had changed from magenta to a paler pink-orange, indicating that the cells had started to metabolise and that the pH of their environment was changing.
  - After the medium change from the previous days, we observed that the cells needed more than 24 hours to double in number. That's why today we decided to check them (via microscopy) at two time points: 25 and 30 hours after the last medium change.
  - At the first time point, we noticed that the number of cells hadn't increased at the rate we anticipated, but their size had definitely increased compared to the previous day.
  - At the second time point, the cells had taken up around 50% of the surface, so we decided to wait longer before we changed their medium.
- **Result Summary**
  - The cells were observed, and no abnormalities were noted.

Pictures in Drive: link

1st timepoint observation



Morning (25 hours post medium change)

2nd timepoint observation



Early afternoon (30 hours post medium change)

MAY 13TH 2026

Overview

- **Date** – Wednesday, May 13th 2026
- **Time (Start–End)** – 7:15 - 9:25
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Melita Lampropoulou
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Medium change for cells.
- **Objective** – The purpose of today's experiment was to check the T-25 flask and change the medium.

Experimental documentation

- **Materials / Reagents Used**
  - ➢ stripettes 5 mL (x2)
  - ➢ DMEM FBS 10% 1% p/s (4,5 mL)
  - ➢ 15 ml Falcon (x1)
- **Method / Protocol Reference**
  - ➢ In-house protocol for changing the medium
- **Sample / Cell Line**
  - ➢ cell line: A-549

Observations & Results

- **Observations / Notes**
  - The color of the medium had changed from magenta to a paler pink-orange, indicating that the cells had started to metabolise and that the pH of their environment was changing.
  - The surface the cells had taken up was around 60%.
- **Result Summary**
  - The cells were observed, and no abnormalities were noted.

Pictures in Drive: link

Before medium change:



After medium change:



MAY 14TH 2026

Overview

- **Date** – Tuesday, May 14th 2026
- **Time (Start–End)** – 10:00 - 10:50 / 16:40 - 17:00
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Alexandros Vlachos and Erriketti Moumka (1st session)
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Observation of cells to determine their exact doubling time.
- **Objective** – The purpose of today's experiment was to check the T-25 flask and find the doubling time of the cells.

Experimental documentation

- **Materials / Reagents Used**
  - ➢ -
- **Method / Protocol Reference**
  - ➢ -
- **Sample / Cell Line**
  - ➢ cell line: A-549

Observations & Results

- **Observations / Notes**
  - The color of the medium had changed from magenta to a paler pink-orange, indicating that the cells had started to metabolise and that the pH of their environment was changing.
  - After the medium change from the previous days, we observed that the cells needed more than 24 hours to double in number. That's why today we decided to check them (via microscopy) at two time points: 24 and 31 hours after the last medium change.
  - At the first time point, we noticed that the number of cells hadn't increased at the rate we anticipated, but their size had definitely increased compared to the previous day.
  - At the second time point, the cells had covered around 60% of the surface, so we decided to wait longer before changing their medium.
- **Result Summary**
  - The cells were observed, and no abnormalities were noted.

Pictures in drive: link

1st observation (24h post medium change)



Morning (24 hours post medium change)

2nd observation



Afternoon (31 hours post medium change)

MAY 15TH 2026

Overview

- **Date** – Friday, May 15th 2026
- **Time (Start–End)** – 7:40 - 9:45
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Melita Lampropoulou
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Medium change for cells, cryopreserving cells in cryovials
- **Objective** – The purpose of today's experiment was to examine the T-25 flask and assess whether cell confluency was sufficient to proceed with the cryopreservation procedure.
    - ⅓ of the total flask volume was used for the reculture process.
    - ⅔ of the total flask volume was used for the making of 1 cryovial.

Experimental documentation

- **Materials / Reagents Used**
    - stripettes 5 mL (x12)
    - DMEM FBS 10% 1% p/s (14 mL)
    - 50 ml Falcon (x1)
    - 15 ml Falcon (x3)
    - PBS (3 mL)
    - Trypsin - EDTA 0,05% (3 mL)
    - Freezing Medium (1 mL)
    - 1.5 mL Eppendorf tube (x1)
    - Trypan Blue (10 μL)
    - 10 μL tips (x2)
    - 1.5 mL cryovials [white cap] (x1)
- **Method / Protocol Reference**
    - In-house protocol for changing the medium
    - In-house protocol for cryopreserving cells.
- **Sample / Cell Line**
    - cell line: A-549

Observations & Results

- **Observations / Notes**
    - The color of the medium had changed from magenta to a paler pink-orange, indicating that the cells had started to metabolise and that the pH of their environment was changing.
    - The surface the cells had taken up was around 80%.
    - After the centrifugation, pellets were present in both Falcon tubes.
    - The cryovial was labelled (A-549, 15/5/26, MP-igem) in more than one place and is now stored in the yellow box (-80 °C) at the molecular laboratory.
- **Result Summary**

- The cells were observed, and no abnormalities were noted.
- The cryopreservation procedure was executed smoothly, within the safe-for-the-cells time slot.

Cell viability Excel: link
Pictures in Drive: link
Before cryovial



BEFORE CHANGE + CRYOVIAL
(this is the density we saw before easter as well)

After cryovial-Reculture with ⅓ of the initial volume



AFTER RECULTURE

MAY 17TH 2026

Overview

- **Date** – Sunday, May 17th 2026
- **Time (Start–End)** – 8:00 - 8:23
- **Name / Initials** – Margarita Papastergiou (MP)
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Medium change for cells.
- **Objective** – The purpose of today's experiment was to check the T-25 flask and change the medium.

Experimental documentation

- **Materials / Reagents Used**
  - ➢ stripettes 5 mL (x2)
  - ➢ DMEM FBS 10% 1% p/s (4,5 mL)
  - ➢ 15 ml Falcon (x1)
- **Method / Protocol Reference**
  - ➢ In-house protocol for changing the medium
- **Sample / Cell Line**
  - ➢ cell line: A-549

Observations & Results

- **Observations / Notes**
  - The color of the medium had changed from magenta to a paler pink-orange, indicating that the cells had started to metabolise and that the pH of their environment was changing.
  - The surface the cells had taken up was around 40%.
  - The total volume of the flask before the reculture process was 4.4 ml, indicating a 0.6 ml reduction (initial volume=5ml). This was the first time we had come across such a reduction in total volume.
- **Result Summary**
  - The cells were observed, and no abnormalities were noted.

Pictures in Drive: link

Before the medium change



BEFORE CHANGE

After the medium change



AFTER CHANGE

MAY 19TH 2026

Overview

- **Date** – Monday, May 19th 2026
- **Time (Start–End)** – 9:25 - 9:50
- **Name / Initials** – Margarita Papastergiou (MP)
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Medium change for cells.
- **Objective** – The purpose of today's experiment was to check the T-25 flask and change the medium.

Experimental documentation

- **Materials / Reagents Used**
  - ➢ stripettes 5 mL (x2)
  - ➢ DMEM FBS 10% 1% p/s (4,5 mL)
  - ➢ 15 ml Falcon (x1)
- **Method / Protocol Reference**
  - ➢ In-house protocol for changing the medium
- **Sample / Cell Line**
  - ➢ cell line: A-549

Observations & Results

- **Observations / Notes**
  - The color of the medium had changed from magenta to a paler pink-orange, indicating that the cells had started to metabolise and that the pH of their environment was changing.
  - The surface the cells had taken up was around 50%.
  - The total volume of the flask before the reculture process was 4.8 ml, indicating a 0.2 ml reduction (initial volume=5ml).

Result Summary

- The cells were observed, and no abnormalities were noted.

Pictures in Drive: link

Before medium change



BEFORE CHANGE

After medium change



AFTER CHANGE

MAY 21ST 2026

Overview

- **Date** – Thursday, May 21st 2026
- **Time (Start–End)** – 9:20 - 9:50
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Alexandros Vlachos and Erriketti Moumka
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Medium change for cells.
- **Objective** – The purpose of today's experiment was to check the T-25 flask and change the medium.

Experimental documentation

- **Materials / Reagents Used**
  - ➢ stripettes 5 mL (x2)
  - ➢ DMEM FBS 10% 1% p/s (4,5 mL)
  - ➢ 15 ml Falcon (x1)
- **Method / Protocol Reference**
  - ➢ In-house protocol for changing the medium
- **Sample / Cell Line**
  - ➢ cell line: A-549

Observations & Results

- **Observations / Notes**
  - The color of the medium had changed from magenta to a paler pink-orange, indicating that the cells had started to metabolise and that the pH of their environment was changing.
  - The surface the cells had taken up was around 50%.

Result Summary

- The cells were observed, and no abnormalities were noted.

Pictures in Drive: link

Before medium change



BEFORE CHANGE

After medium change



AFTER CHANGE

MAY 23rd 2026

Overview

- **Date** – Saturday, May 23rd 2026
- **Time (Start–End)** – 9:10 - 10:20
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Erriketti Moumka, Alexandros Vlachos
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Medium change for cells, cryopreserving cells in cryovials
- **Objective** – The purpose of today's experiment was to examine the T-25 flask and assess whether cell confluency was sufficient to proceed with the cryopreservation procedure.
    - ⅓ of the total flask volume was used for the reculture process.
    - ⅔ of the total flask volume was used for the making of 1 cryovial.

Experimental documentation

- **Materials / Reagents Used**
    - stripettes 5 mL (x12)
    - DMEM FBS 10% 1% p/s (14 mL)
    - 50 ml Falcon (x1)
    - 15 ml Falcon (x3)
    - PBS (3 mL)
    - Trypsin - EDTA 0,05% (3 mL)
    - Freezing Medium (1 mL)
    - 1.5 mL Eppendorf tube (x1)
    - Trypan Blue (10 μL)
    - 10 μL tips (x2)
    - 1.5 mL cryovials [white cap] (x1)
- **Method / Protocol Reference**
    - In-house protocol for changing the medium
    - In-house protocol for cryopreserving cells.
- **Sample / Cell Line**
    - cell line: A-549

Observations & Results

- **Observations / Notes**
    - The color of the medium had changed from magenta to a paler pink-orange, indicating that the cells had started to metabolise and that the pH of their environment was changing.
    - The surface the cells had taken up was around 80%.
    - After the centrifugation, pellets were present in both Falcon tubes.
    - The cryovial was labelled (A-549, 15/5/26, MP-igem) in more than one place and is now stored in the yellow box (-80 °C) at the molecular laboratory.
- **Result Summary**

- The cells were observed, and no abnormalities were noted.
- The cryopreservation procedure was executed smoothly, within the safe-for-the-cells time slot.

Cell viability Excel: link
Pictures in Drive: link
Before cryovial



BEFORE CHANGE + CRYOVIAL

After cryovial-Reculture with ⅓ of the initial volume

MAY 25TH 2026

Overview

- **Date** – Monday, May 25th 2026
- **Time (Start–End)** – 9:20 - 9:50
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Erriketti Moumka
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Medium change for cells.
- **Objective** – The purpose of today's experiment was to check the T-25 flask and change the medium.

Experimental documentation

- **Materials / Reagents Used**
  - ➢ stripettes 5 mL (x2)
  - ➢ DMEM FBS 10% 1% p/s (4,5 mL)
  - ➢ 15 ml Falcon (x1)
- **Method / Protocol Reference**
  - ➢ In-house protocol for changing the medium
- **Sample / Cell Line**
  - ➢ cell line: A-549

Observations & Results

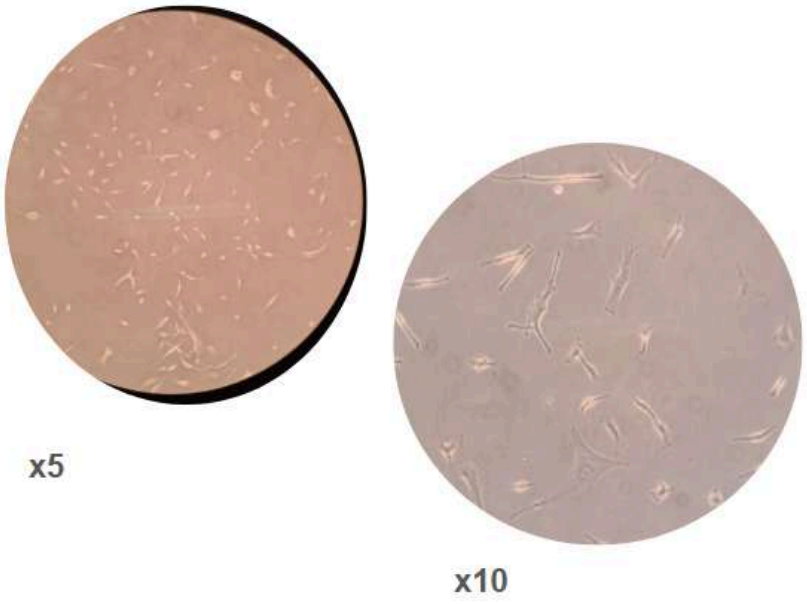
- **Observations / Notes**
  - The color of the medium had changed from magenta to a paler pink-orange, indicating that the cells had started to metabolise and that the pH of their environment was changing.
  - The surface the cells had taken up was around 50%.

Result Summary

- The cells were observed, and no abnormalities were noted.

Pictures in Drive: link

Before medium change



BEFORE CHANGE

After medium change



AFTER CHANGE

MAY 27TH 2026

Overview

- **Date** – Wednesday, May 27th 2026
- **Time (Start–End)** – 9:00 - 10:30
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Erriketti Moumka, Anna Filindri
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Cell culture medium preparation, medium change for cells
- **Objective** – The purpose of today's experiment was to prepare cell culture medium for the A-549 cell line. Also, the T-25 flask was observed, and the medium was changed.

Experimental documentation

- **Materials / Reagents Used**
  - ➢ stripettes 5 mL (x9)
  - ➢ stripettes 10 mL (x2)
  - ➢ DMEM FBS 10% 1% p/s (4,5 mL)
  - ➢ 15 ml Falcon (x1)
  - ➢ 50 ml Falcon (x4)
  - ➢ Sterile syringe 10 mL (x1)
  - ➢ Sterile filter for syringe (x2)
  - ➢ Pen/strep (1 mL)
  - ➢ FBS (10 mL)
  - ➢ DMEM (89 mL)
- **Method / Protocol Reference**
  - ➢ In-house protocol for changing the medium
  - ➢ In-house protocol for cell culture medium preparation
- **Sample / Cell Line**
  - ➢ cell line: A-549

Observations & Results

- **Observations / Notes**
  - The color of the medium had changed from a magenta color to a more pale pink-orange color, indicating that the cells had started to metabolise and the pH of their environment was changing.
  - The surface the cells had taken up was around 55%.
- **Result Summary**
  - Fresh cell culture medium was prepared.
  - The cells were observed, and no abnormalities were noted.

Pictures in drive: link

Before medium change

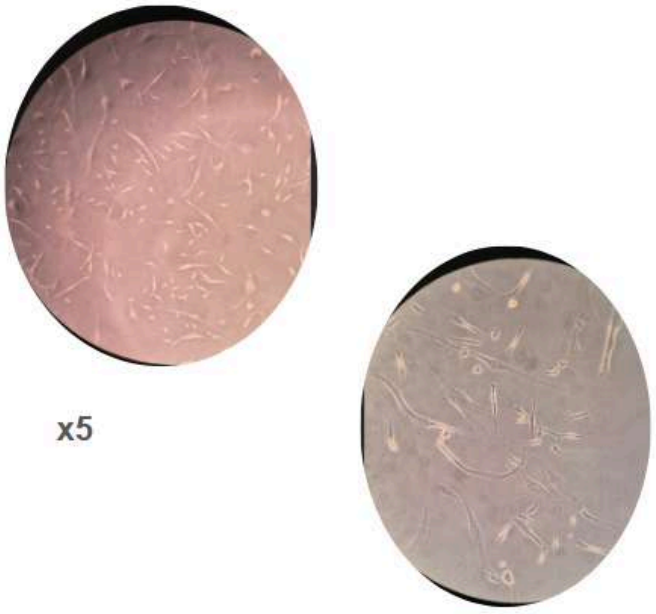


x5

x10

BEFORE CHANGE

After medium change



x5

AFTER CHANGE

x10

MAY 29TH 2026

Overview

- **Date** – Friday, May 29th 2026
- **Time (Start–End)** – 9:00 - 9:20
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Erriketti Moumka
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Medium change for cells.
- **Objective** – The purpose of today's experiment was to check the T-25 flask and change the medium.

Experimental documentation

- **Materials / Reagents Used**
  - ➢ stripettes 5 mL (x2)
  - ➢ DMEM FBS 10% 1% p/s (4,5 mL)
  - ➢ 15 ml Falcon (x1)
- **Method / Protocol Reference**
  - ➢ In-house protocol for changing the medium
- **Sample / Cell Line**
  - ➢ cell line: A-549

Observations & Results

- **Observations / Notes**
  - The color of the medium had changed from magenta to a paler pink-orange, indicating that the cells had started to metabolise and that the pH of their environment was changing.
  - The surface the cells had taken up was around 60%.

Result Summary

- The cells were observed, and no abnormalities were noted.

Pictures in drive: link

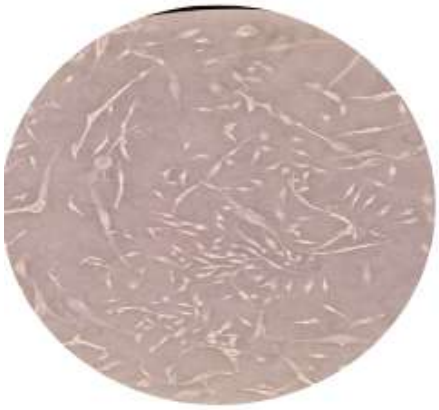
Before medium change



x10

BEFORE CHANGE

After medium change



x5



AFTER CHANGE

x10

MAY 31ST 2026

Overview

- **Date** – Saturday, May 31st 2026
- **Time (Start–End)** – 9:15 - 12:45
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Erriketti Moumka, Melita Lampropoulou
- **Project / Subproject** – A-549 Cell Seeding Optimization: Cell Density Titration and Morphology Assessment Over 72 Hours
- **Experiment / Task Title** – Medium change for cells and cell seeding for 3 different conditions (10k cells/well, 20k cells/well, 30k cells/well).
- **Objective** – The purpose of today's experiment was to check the T-25 flask and change the medium, as well as perform the cell seeding for the titration of cells/well in a 24-well plate.

Experimental documentation

- **Materials / Reagents Used**
  - ➢ stripettes 5 mL (x7)
  - ➢ DMEM FBS 10% 1% p/s (12,1 mL)
  - ➢ 15 ml Falcon (x1)
  - ➢ 30 ml Falcon (x1)
  - ➢ 24-well plate (x1)
  - ➢ 1.5 ml tubes (x8)
  - ➢ PBS (2 ml)
  - ➢ Trypsin - EDTA (0.05%) (2 ml)
  - ➢ Trypan Blue (50 µL)
  - ➢ 10 µL tips (x10)
  - ➢ 1000 µL tips (x10)
- **Method / Protocol Reference**
  - ➢ In-house protocol for changing the medium
  - ➢ Cell seeding protocol for A-549 cells - Titration edition
- **Sample / Cell Line**
  - ➢ cell line: A-549

Observations & Results - day 0:

Before cell seeding:

- The color of the medium had changed from magenta to a paler pink-orange, indicating that the cells had started to metabolise and that the pH of their environment was changing.
- The surface the cells had taken up was around 70%.



Figure x: Representative image under microscope, A-549 cells in 5x focus (left) and 10x focus (right), before medium change

During cell seeding:

- After thorough pipetting up and down to homogenize the cell suspension, an aliquot was taken for Trypan Blue staining and cell counting using a Neubauer chamber. However, no cells or only very few cells were observed in the chamber, making the cell count unreliable.
- To overcome this issue, the tubes containing the cell suspension were centrifuged to pellet the cells. Most of the supernatant was discarded, leaving approximately 40 µL behind for resuspension of the cell pellet. As a result, the cell suspension became more concentrated and was no longer representative of the original cell concentration in the flask.
- Following resuspension, the cells were stained with Trypan Blue and loaded into the Neubauer chamber for counting. However, the change in cell concentration caused by the centrifugation and removal of supernatant was not taken into account when calculating the volume of cell suspension required for the different seeding conditions.
- Therefore, the number of cells seeded per well was likely lower than the intended/expected number.

Before centrifugation: 240 µL in the tube
After centrifugation and resuspension: 40 µL in the tube
So the cells were concentrated by a factor of 240/40=**6**

- This means that the suspension loaded into the Neubauer chamber after centrifugation was 6 times more concentrated than the original suspension taken directly from the flask.

Cells/mL in the concentrated sample = 310.000 cells/mL

Cells/mL in the original 240 µL and flask = 310.000 / 6 = 51.667 cells/mL

Conditions (x2 for the 2 TRs)	Volume that was **taken** from the flask (µL)	Volume that **should** have been taken from the flask (µL)
10.000 cells/well	65	82,3
20.000 cells/well	130	164
30.000 cells/well	200	247

After cell seeding:

- After the seeding process, bubbles were present in some wells.
- The wells were observed, and cells were present.
- No abnormalities were noted.

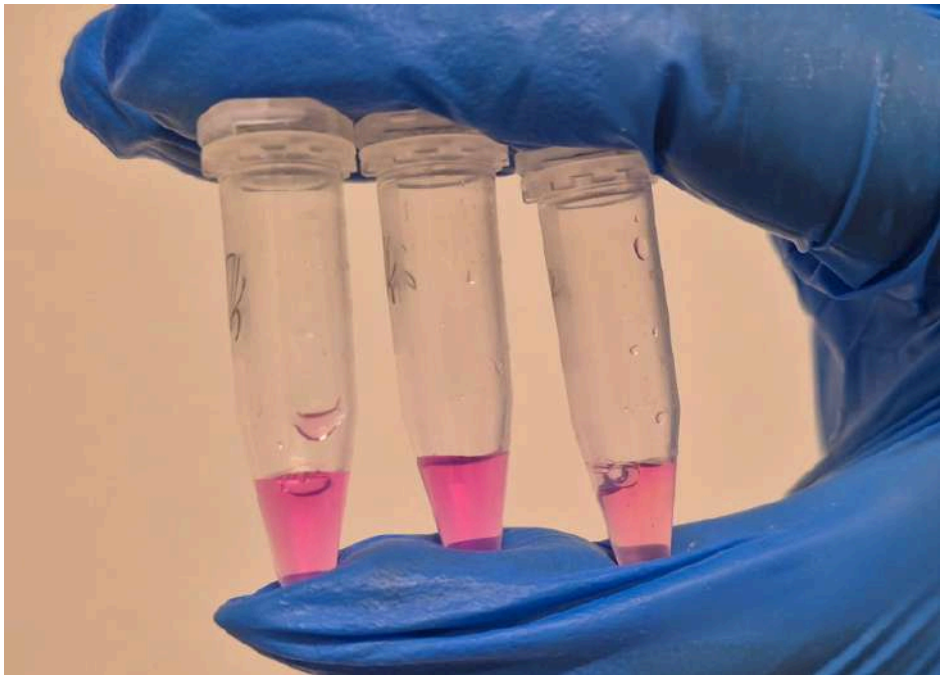


Figure x: Representative image of 1.5 mL tubes after collecting A-549 cells for the seeding process. The same volume (200 µL) can be observed, indicating that the volumes shared were most likely correct.

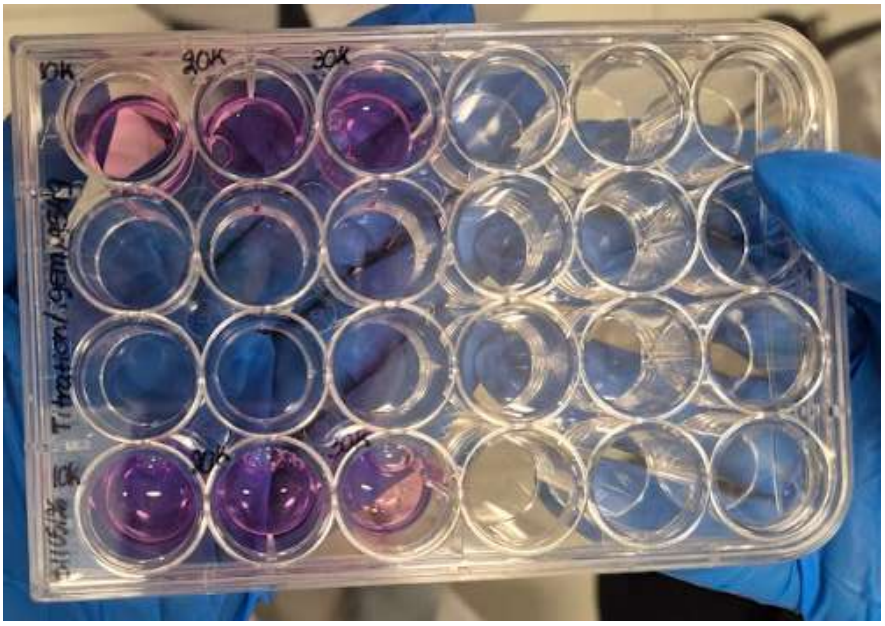


Figure x: Representative image of plate immediately after seeding A-549 cells. Different conditions are shown. The two technical replicates are shown, as well: TR1 (up), TR2 (down)

JUNE 1ST 2026

Overview

- **Date** – Monday, June 1st 2026
- **Time (Start–End)** – 9:00 - 10:30
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Melita Lampropoulou
- **Project / Subproject** – A-549 Cell Seeding Optimization: Cell Density Titration and Morphology Assessment Over 72 Hours
- **Experiment / Task Title** – Observation of cell density 24h post-seeding.
- **Objective** – The purpose of today's experiment was to observe the different cell densities of A-549 cells, 24 hours post-seeding.

Experimental documentation

- **Materials / Reagents Used**
  - ❖ Inverted microscope
- **Method / Protocol Reference**
  - ➢ Cell seeding protocol for A-549 cells - Titration edition
- **Sample / Cell Line**
  - ➢ cell line: A-549

Observations & Results - day 1 [titration vol.1]

- The color of the medium had changed from magenta to a paler pink-orange, indicating that the cells had started to metabolise and that the pH of their environment was changing.
- The cells had mostly taken up the perimeter of the wells, avoiding the center, except in the 20k cells/well condition, where cells were mostly at the center of the well.
- The bubbles that were created the day prior had disappeared.
- The technical replicates showed consistent morphology and no major observable differences during microscopic assessment.

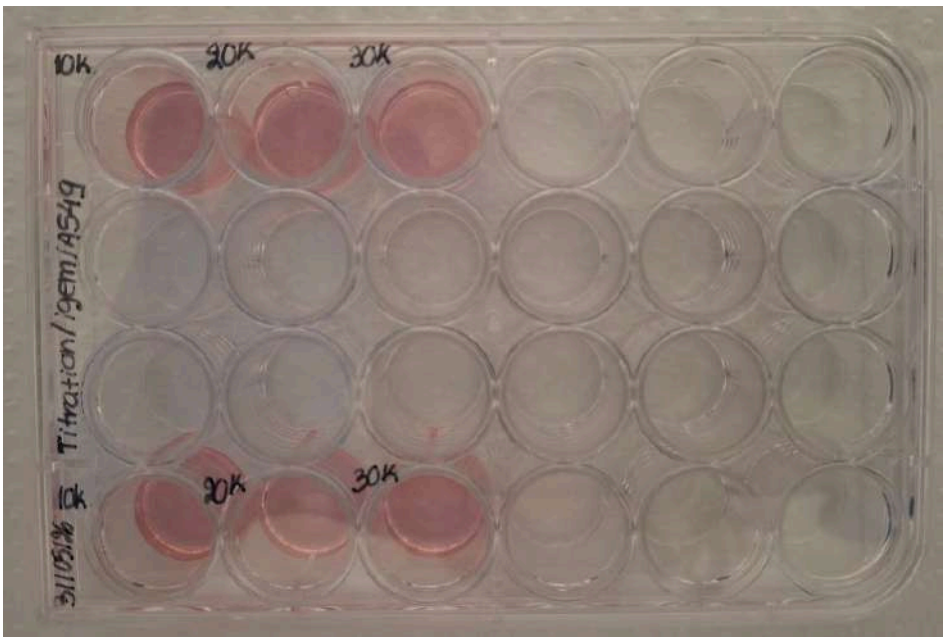


Figure x: Representative picture of the 24-well plate 24 hours post cell-seeding.

JUNE 2ND 2026

Overview

- **Date** – Tuesday, June 2nd 2026
- **Time (Start–End)** – 8:30 - 13:00
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Melita Lampropoulou, Erriketti Moumka
- **Project / Subproject** – A-549 Cell Seeding Optimization: Cell Density Titration and Morphology Assessment Over 72 Hours / A-549 Cell Seeding Optimization: Cell Density Titration and Morphology Assessment Over 72 Hours (vol. 2)
- **Experiment / Task Title** – Observation of cell density 24h post-seeding, cell seeding for 3 different conditions (10k cells/well, 20k cells/well, 30k cells/well) [vol.2], change of medium and transition to a T-75 flask.
- **Objective** – The purpose of today's experiment was to observe the different cell densities of A-549 cells, 24 hours post-seeding, and repeat the cell seeding process for the same 3 different conditions. Lastly, the medium of the cells was changed as well as their flask (T-75).

Experimental documentation

- **Materials / Reagents Used**
  - ❖ stripettes 5 mL (x9)
  - ❖ DMEM FBS 10% 1% p/s (10,2 mL)
  - ❖ 15 ml Falcon (x1)
  - ❖ 30 ml Falcon (x1)
  - ❖ 1.5 ml tubes (x6)
  - ❖ PBS (2 ml)
  - ❖ Trypsin - EDTA (0.05%) (2 ml)
  - ❖ Trypan Blue (50 µL)
  - ❖ 10 µL tips (x10)
  - ❖ 1000 µL tips (x10)
  - ❖ T-75 flask
  - ❖ Inverted microscope
- **Method / Protocol Reference**
  - ➢ In-house protocol for changing the medium
  - ➢ Cell seeding protocol for A-549 cells - Titration edition
- **Sample / Cell Line**
  - ➢ cell line: A-549

Observations & Results - day 2 [titration vol.1] and day 0 [titration vol.2]

Day 2 [titration vol.1]

- The color of the medium had changed from magenta to a paler pink-orange, indicating that the cells had started to metabolise and that the pH of their environment was changing.

- The cells had mostly taken up the perimeter of the wells, avoiding the center, except in the 20k cells/well condition, which had cells mostly at the center of the well.
- The bubbles that were created the day prior had disappeared.
- The technical replicates showed consistent morphology and no major observable differences during microscopic assessment.

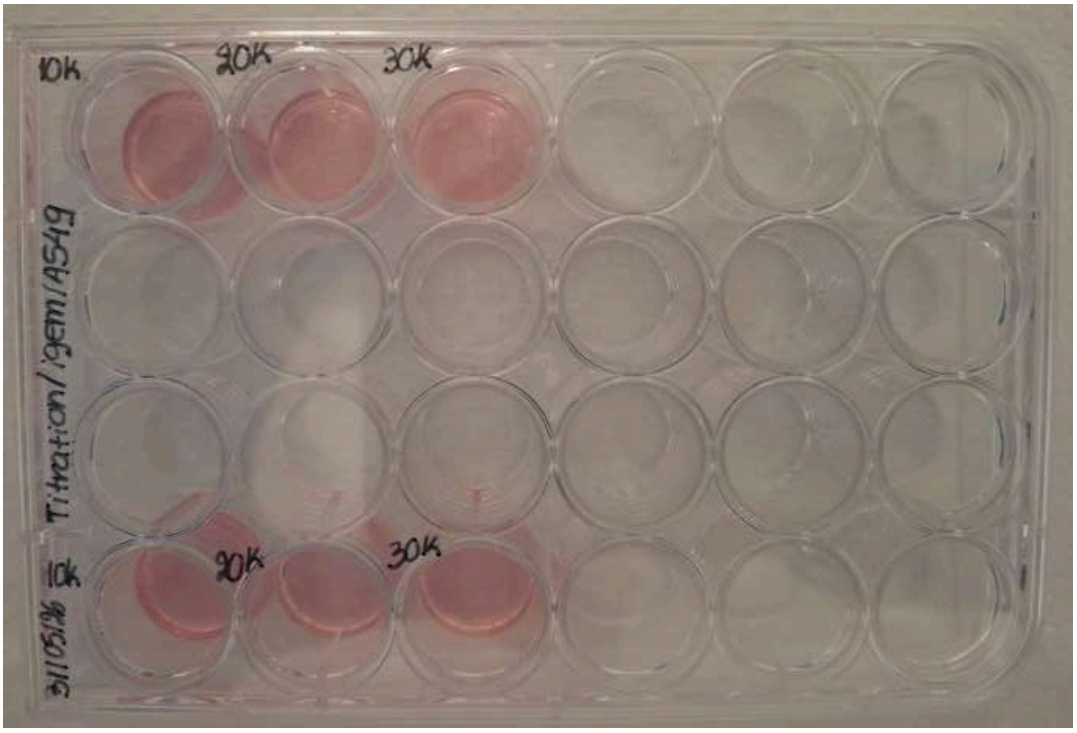
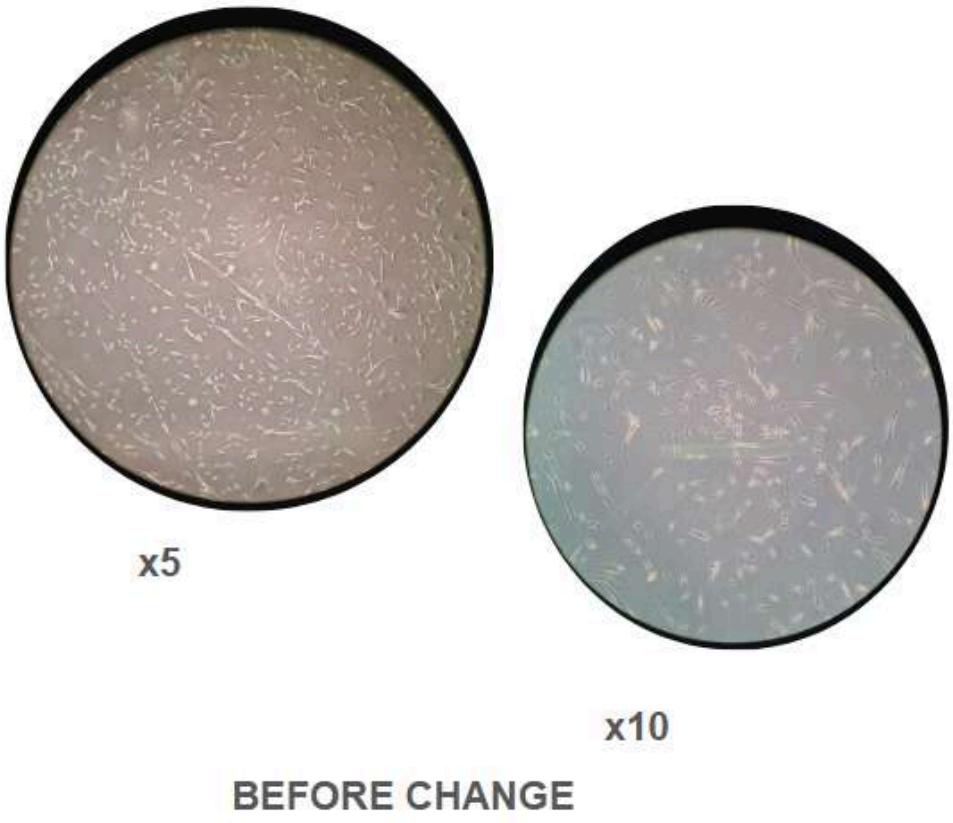


Figure x: Representative picture of the 24-well plate 48 hours post cell-seeding.

Day 0 [titration vol.2]

Before cell seeding

- The color of the medium had changed from magenta to a paler pink-orange, indicating that the cells had started to metabolise and that the pH of their environment was changing.
- The surface the cells had taken up was around 70%.



During cell seeding:

- After thorough pipetting up and down to homogenize the cell suspension, an aliquot of 1mL was taken for Trypan Blue staining and cell counting using a Neubauer chamber.
- After the necessary calculations, the following volumes were taken from the cell suspension:

Condition (x2 for the 2 TRs)	**Volume of cell suspension**
10.000 cells/well	181 µL
20.000 cells/well	363 µL
30.000 cells/well	545 µL

- After the volumes were collected and distributed from the cell suspension into 3 different tubes, the remaining volume was used for the reculture and transfer of the cell culture to a T-75 flask.
- The tubes and the flacon were centrifuged. For the reculture process, the typical protocol was followed, and the final volume of the T-75 flask was 7 mL. For the tubes, the supernatant was removed very carefully, and the pellet was resuspended with 600 µL of full medium and divided into 300 µL for each condition. Then the tube was washed with 600 µL of full medium to ensure

that no cells were left behind, and 200 µL was seeded into each condition. This gave a final volume of 500 µL per well.

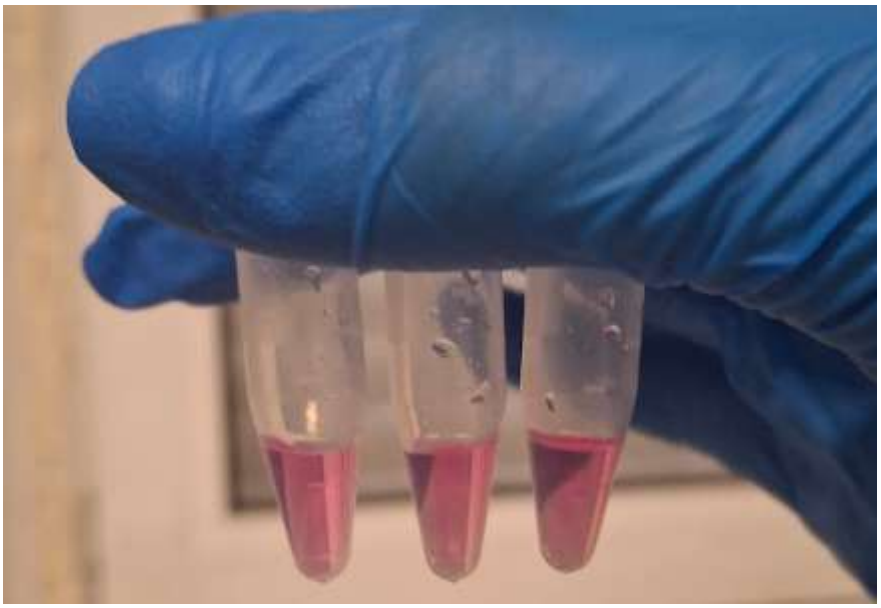


Figure x: The volume left to each tube after the end of the seeding process (300 µL). From left to right: tube corresponding to the 10.000 cells/well, 20.000 cells/well, and 30.000 cells/well.

After cell seeding:

- After the seeding process, some bubbles were present in the TR1 10k cells/well condition.
- The wells were observed, and cells were present.
- No abnormalities were noted.

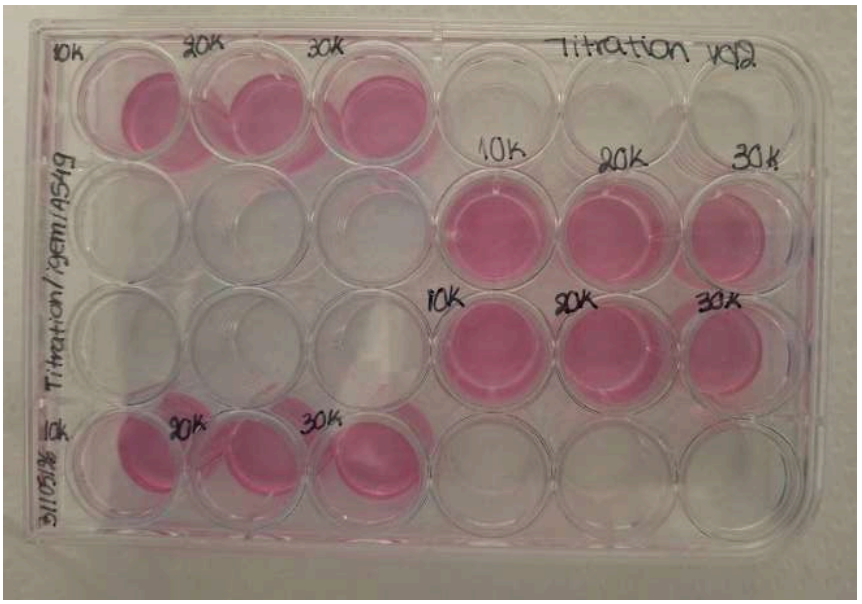


Figure X: Representative image of plate immediately after seeding A-549 cells (vol.2 - right side of plate). Different conditions are shown. The two technical replicates are shown, as well: TR1 (up), TR2 (down)

JUNE 3RD 2026

Overview

- **Date** – Wednesday, June 3rd 2026
- **Time (Start–End)** – 9:20 - 10:00
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Melita Lampropoulou, Erriketti Moumka
- **Project / Subproject** – A-549 Cell Seeding Optimization: Cell Density Titration and Morphology Assessment Over 72 Hours [vol.1 and vol.2]
- **Experiment / Task Title** – Observation of cell density 72 h post-seeding [vol.1] and 24h post-seeding [vol.2].
- **Objective** – The purpose of today's experiment was to observe the different cell densities of A-549 cells, 24 hours post-seeding.

Experimental documentation

- **Materials / Reagents Used**
  - ❖ Inverted microscope
- **Method / Protocol Reference**
  - ➢ Cell seeding protocol for A-549 cells - Titration edition
- **Sample / Cell Line**
  - ➢ cell line: A-549

Observations & Results - day 3 [titration vol.1] and day 1 [titration vol.2]

- The color of the medium had changed from magenta to a paler pink-orange, indicating that the cells had started to metabolise and that the pH of their environment was changing.
- The cells had mostly taken up the perimeter of the wells, avoiding the center, except in the 20k cells/well condition, where cells were mostly at the center of the well.
- The bubbles that were created the day prior had disappeared.
- The technical replicates showed consistent morphology and no major observable differences during microscopic assessment.

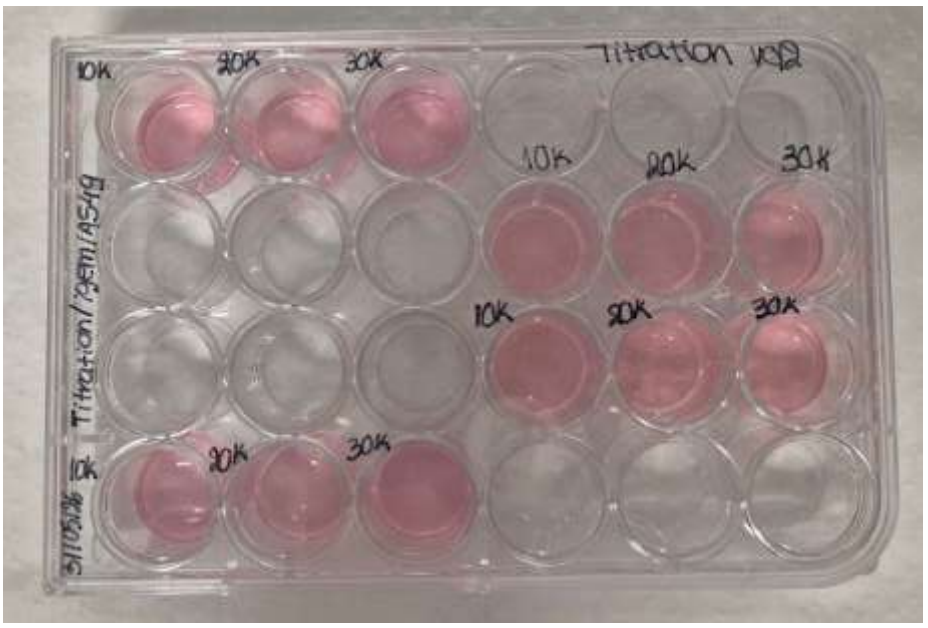


Figure x: Representative image of the 24-well plate, 72 h and 24 h after seeding A-549 cells (vol.2 - right side of plate / vol.1 - left side of plate). Different conditions are shown. The two technical replicates are shown, as well: TR1 (up), TR2 (down)

JUNE 4RTH 2026

Overview

- **Date** – Thursday, June 4th 2026
- **Time (Start–End)** – 9:10 - 11:10
- **Name / Initials** – Margarita Papastergiou (MP), Erriketti Moumka (EM)
- **Shadowing** - Melita Lampropoulou
- **Project / Subproject** – A-549 Cell Seeding Optimization: Cell Density Titration and Morphology Assessment Over 72 Hours [vol.2]
- **Experiment / Task Title** – Observation of cell density 48 h post-seeding [vol.2].
- **Objective** – The purpose of today's experiment was to observe the different cell densities of A-549 cells, 48 hours post-seeding, and reculture (EM).

Experimental documentation

- **Materials / Reagents Used**
  - ❖ Inverted microscope
  - ❖ stripettes 5 mL (x1)
  - ❖ stripettes 10 mL (x2)
  - ❖ DMEM FBS 10% 1% p/s (11 mL)
  - ❖ 30 ml Falcon (x2)
  - ❖ 1000 µL tips (x6)
- **Method / Protocol Reference**
  - ➢ In-house protocol for changing the medium
  - ➢ Cell seeding protocol for A-549 cells - Titration edition
- **Sample / Cell Line**
  - ➢ cell line: A-549

Observations & Results

Day 2 [titration vol.2]

- The color of the medium had changed from magenta to a paler pink-orange, indicating that the cells had started to metabolise and that the pH of their environment was changing.
- The cells had mostly taken up the perimeter of the wells, avoiding the center, except in the 20k cells/well condition, where cells were mostly at the center of the well.
- The technical replicates showed consistent morphology and no major observable differences during microscopic assessment.
- After the observation, we concluded that the conditions of 10k cells/well and 30k cells/well should be **discarded** since their coverage was not as expected and the medium for the 2 TR of the 20k cells/well was changed, with the final volume of each well being 500 µL.

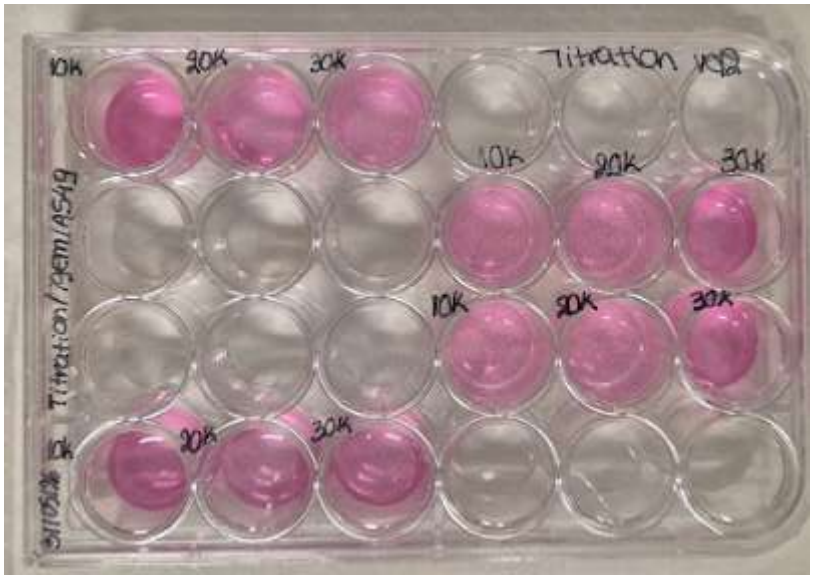


Figure x: Representative image of the 24-well plate, 48 h after seeding A-549 cells (vol.2 - right side of plate). Different conditions are shown. The two technical replicates are shown, as well: TR1 (up), TR2 (down)

Reculture

Observation

- The color of the medium had changed from magenta to a paler pink-orange, indicating that the cells had started to metabolise and that the pH of their environment was changing. Culture volume had changed from 7ml to 6.1 ml of medium.
- The surface the cells had taken up was around 20%

Results

- The cells were observed and no abnormalities were noted
- The cells were recultured in 10 ml of growth medium

Photos in drive: link



JUNE 5TH 2026

Overview

- **Date** – Friday, June 5th 2026
- **Time (Start–End)** – 9:10- 9:45
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Melita Lampropoulou, Erriketti Moumka
- **Project / Subproject** – A-549 Cell Seeding Optimization: Cell Density Titration and Morphology Assessment Over 72 Hours (vol. 2)
- **Experiment / Task Title** – Observation of cell density 72 h post-seeding.
- **Objective** – The purpose of today's experiment was to observe the different cell densities of A-549 cells, 72 hours post-seeding.

Experimental documentation

- **Materials / Reagents Used**
  - ❖ Inverted microscope
- **Method / Protocol Reference**
  - ➢ Cell seeding protocol for A-549 cells
- **Sample / Cell Line**
  - ➢ cell line: A-549

Observations & Results - day 3 [titration vol.1]

- The color of the medium had changed from magenta to a paler pink-orange, indicating that the cells had started to metabolise and that the pH of their environment was changing.
- The cells had mostly taken up the perimeter of the wells, avoiding the center, except in the 20k cells/well condition, where cells were mostly at the center of the well.
- The bubbles that were created the day prior had disappeared.
- The technical replicates showed consistent morphology and no major observable differences during microscopic assessment.
- The cells were more present in the condition of 20k cells/well.

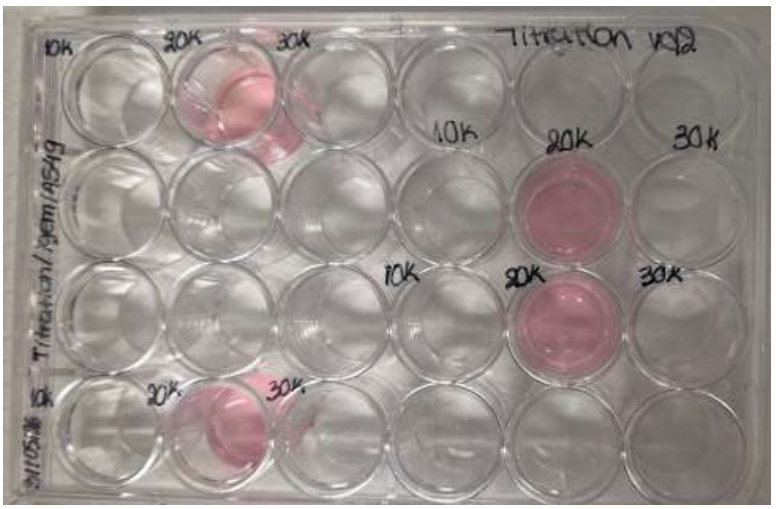


Figure x: Representative image of the 24-well plate, 72 h after seeding A-549 cells (vol.2 - right side of plate). Different conditions are shown. The two technical replicates are shown, as well: TR1 (up), TR2 (down)

JUNE 6TH 2026

Overview

- **Date** – Saturday, June 6th 2026
- **Time (Start–End)** – 9:10 - 9:45
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Melita Lampropoulou, Alexandros Vlachos
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Cell observation of T-75 flask.
- **Objective** – The purpose of today's experiment was to observe how the A-549 cells are adjusting to the new T-75 flask, and assess where or not the medium needs to be changed.

Experimental documentation

- **Materials / Reagents Used**
  - ❖ Inverted microscope
- **Sample / Cell Line**
  - ➢ cell line: A-549

Observations & Results

- The color of the medium had not changed from magenta to a paler pink-orange, indicating that the cells had not started to metabolise and that the pH of their environment had not changed.
- For that reason, we decided to further stress them by changing the medium.
- The flask was returned to the incubator without any other procedures being done.

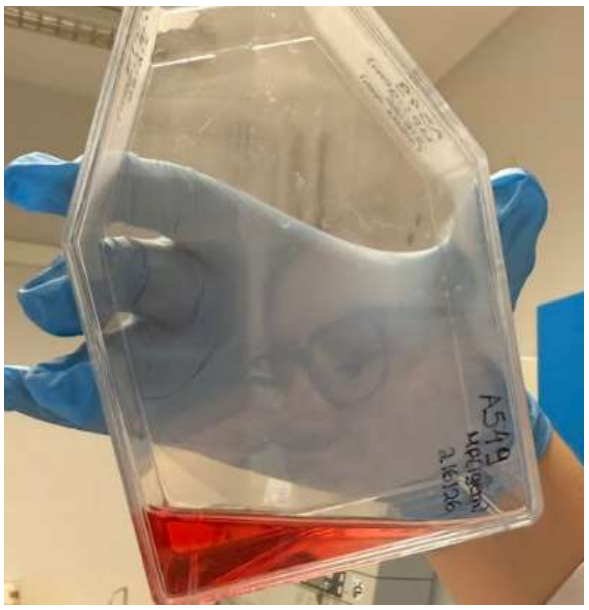


Figure x: Representative picture of the T-75 flask, showing that there was no change in the color of the medium.

JUNE 7TH 2026

Overview

- **Date** – Sunday, June 7th 2026
- **Time (Start–End)** – 9:00 - 9:40
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Melita Lampropoulou, Alexandros Vlachos
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Medium change for cells.
- **Objective** – The purpose of today's experiment was to check the T-75 flask and change the medium.

Experimental documentation

- **Materials / Reagents Used**
  - ➢ Inverted microscope
  - ➢ stripettes 10 mL (x2)
  - ➢ DMEM FBS 10% 1% p/s (9 mL)
  - ➢ 30 ml Falcon (x1)
- **Method / Protocol Reference**
  - ➢ In-house protocol for changing the medium
- **Sample / Cell Line**
  - ➢ cell line: A-549

Observations & Results

- **Observations / Notes**
  - The color of the medium had changed from magenta to a paler pink-orange, indicating that the cells had started to metabolise and that the pH of their environment was changing.
  - The surface the cells had taken up was around 50%.

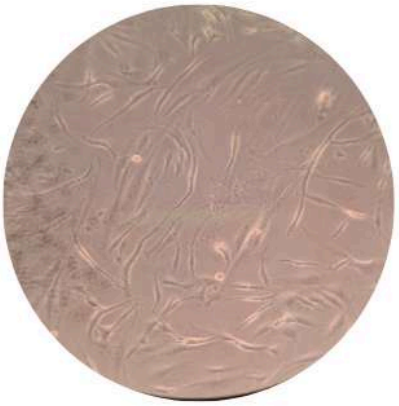
Result Summary

- The cells were observed, and no abnormalities were noted.

Pictures in drive: link



x5



x10

JUNE 9TH 2026

Overview

- **Date** – Tuesday, June 9th 2026
- **Time (Start–End)** – 9:00 - 9:50
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Alexandros Vlachos
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Medium change for cells.
- **Objective** – The purpose of today's experiment was to check the T-75 flask and change the medium.

Experimental documentation

- **Materials / Reagents Used**
  - ➢ Inverted microscope
  - ➢ stripettes 10 mL (x2)
  - ➢ DMEM FBS 10% 1% p/s (9 mL)
  - ➢ 30 ml Falcon (x1)
- **Method / Protocol Reference**
  - ➢ In-house protocol for changing the medium
- **Sample / Cell Line**
  - ➢ cell line: A-549

Observations & Results

- **Observations / Notes**
  - The color of the medium had changed from magenta to a paler pink-orange, indicating that the cells had started to metabolise and that the pH of their environment was changing.
  - The surface the cells had taken up was around 50%.

Result Summary

- The cells were observed, and no abnormalities were noted.

Pictures in drive: link



x5



x10

JUNE 11TH 2026

Overview

- **Date** – Thursday, June 11th 2026
- **Time (Start–End)** – 11:30 - 12:10
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Alexandros Vlachos, Erriketti Moumka
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Medium change for cells.
- **Objective** – The purpose of today's experiment was to check the T-75 flask and change the medium.

Experimental documentation

- **Materials / Reagents Used**
  - ➢ Inverted microscope
  - ➢ stripettes 10 mL (x2)
  - ➢ DMEM FBS 10% 1% p/s (9 mL)
  - ➢ 30 ml Falcon (x1)
- **Method / Protocol Reference**
  - ➢ In-house protocol for changing the medium
- **Sample / Cell Line**
  - ➢ cell line: A-549

Observations & Results

- **Observations / Notes**
  - The color of the medium had changed from magenta to a paler pink-orange, indicating that the cells had started to metabolise and that the pH of their environment was changing.
  - The surface the cells had taken up was around 55%.

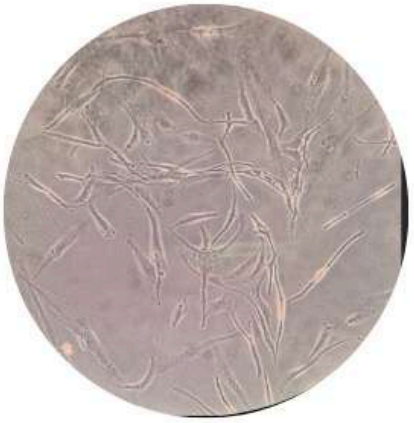
Result Summary

- The cells were observed, and no abnormalities were noted.

Pictures in drive: link



x5



x10

JUNE 13TH 2026

Overview

- **Date** – Saturday, June 13th 2026
- **Time (Start–End)** – 9:05 - 11:45
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Erriketti Moumka, Alexandros Vlachos
- **Project / Subproject** – A-549 Cell Seeding Optimization: Cell Density Titration and Morphology Assessment Over 72 Hours [vol. 3]
- **Experiment / Task Title** – Medium change for cells and cell seeding for two conditions with 3 technical replicates (40k cells/well & 25k cells/well).
- **Objective** – The purpose of today's experiment was to check the T-75 flask and change the medium, as well as perform the cell seeding for the titration of cells/well in a 24-well plate and 96-well plate.

Experimental documentation

- **Materials / Reagents Used**
  - ➢ stripettes 10 mL (x5)
  - ➢ stripettes 5 mL (x3)
  - ➢ DMEM FBS 10% 1% p/s (15 mL)
  - ➢ 15 ml Falcon (x1)
  - ➢ 30 ml Falcon (x1)
  - ➢ 24-well plate (x1)
  - ➢ 96-well plate (x1)
  - ➢ 1.5 ml tubes (x5)
  - ➢ PBS (3 ml)
  - ➢ Trypsin - EDTA (0.05%) (1,5 ml)
  - ➢ Trypan Blue (30 μL)
  - ➢ 10 μL tips (x6)
  - ➢ 1000 μL tips (x8)
- **Method / Protocol Reference**
  - ➢ In-house protocol for changing the medium
  - ➢ Cell seeding protocol for A-549 cells - Titration edition
- **Sample / Cell Line**
  - ➢ cell line: A-549

Observations & Results - day 0:

Before cell seeding:

- The color of the medium had changed from magenta to a paler pink-orange, indicating that the cells had started to metabolise and that the pH of their environment was changing.
- The surface the cells had taken up was around 70%.



Figure x: Representative image under microscope, A-549 cells in 5x focus (left) and 10x focus (right), before medium change and cell seeding.

During cell seeding:

- After thorough pipetting up and down to homogenize the cell suspension, an aliquot of 1 mL was taken from the flask, from which 20 μL were taken for Trypan Blue staining and cell counting using a Neubauer chamber.
- After the cell counting process and calculations, the concentration in the flask was 70.000 cells/mL.
- The volumes taken from the flask were: 1.71 mL for the 3 TRs of the 24-well plate (40k cells/well) and 1.1 mL for the 3 TRs of the 96-well plate (25k cells/well) [the 980 μL left from the 1mL aliquot were used as well for the 24-well plate needed volume].
- The corresponding volumes for each condition were collected from the T-75 flask and centrifuged at 1200 rpm for 5 min. After the centrifuge step, pellets were present in all tubes.
- During the seeding process at the TR1 of the 24-well plate, up and down was performed at a milder pace compared to the other 2 TR, leading to a slightly different cell density that was observed at the overview of the plate.

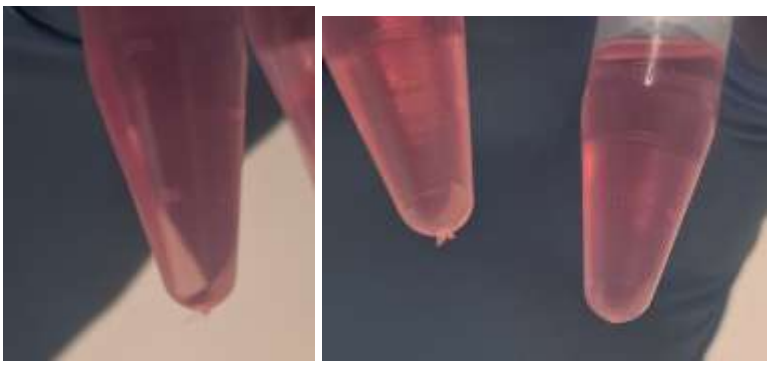


Figure x: Pictures of tubes after the centrifuging step. Pellets are present in all of them.

After cell seeding:

- After the seeding process, bubbles were present in some wells.
- The wells were observed, and cells were present.
- No abnormalities were noted.

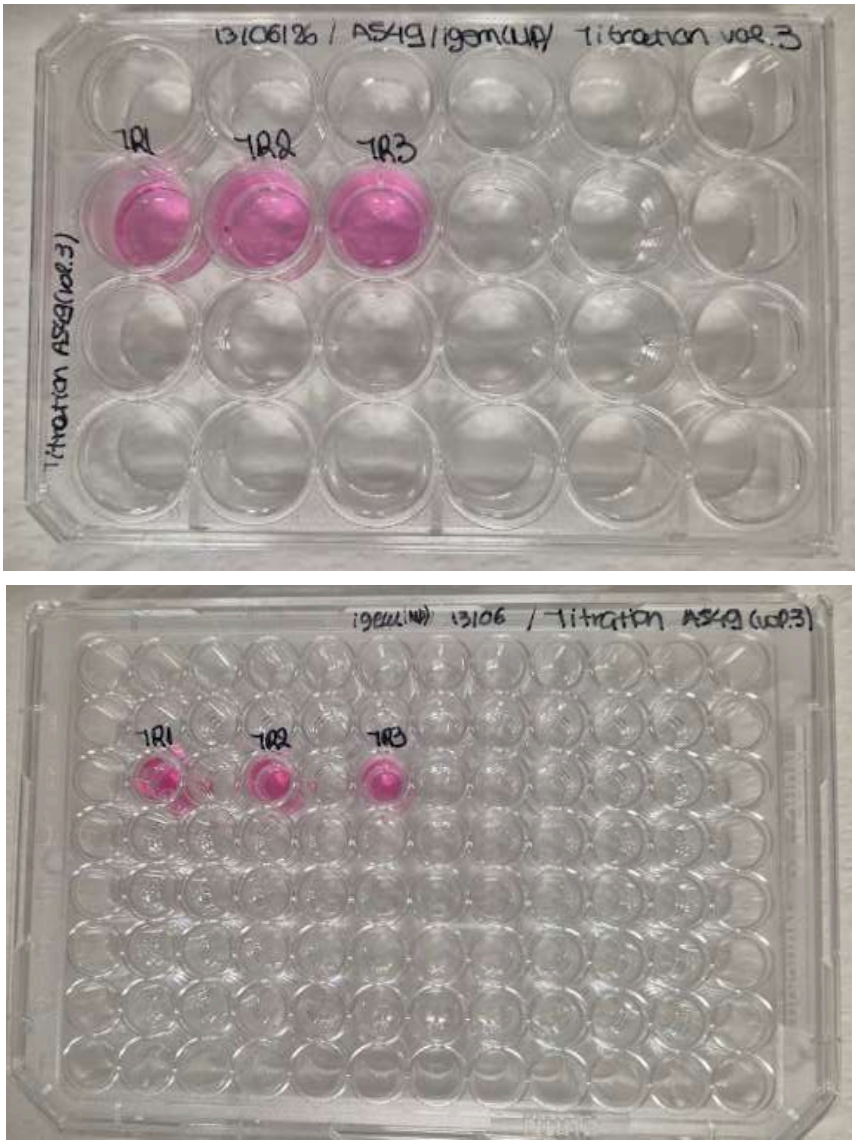


Figure x: Representative image of the 2 plates immediately after seeding A-549 cells. Different conditions are shown (40k cells/well -> 24 well-plate, 25k cells/well -> 96-well plate). The 3 technical replicates are shown, as well: TR1 (left), TR2 (middle), TR3 (right)

JUNE 14TH 2026

Overview

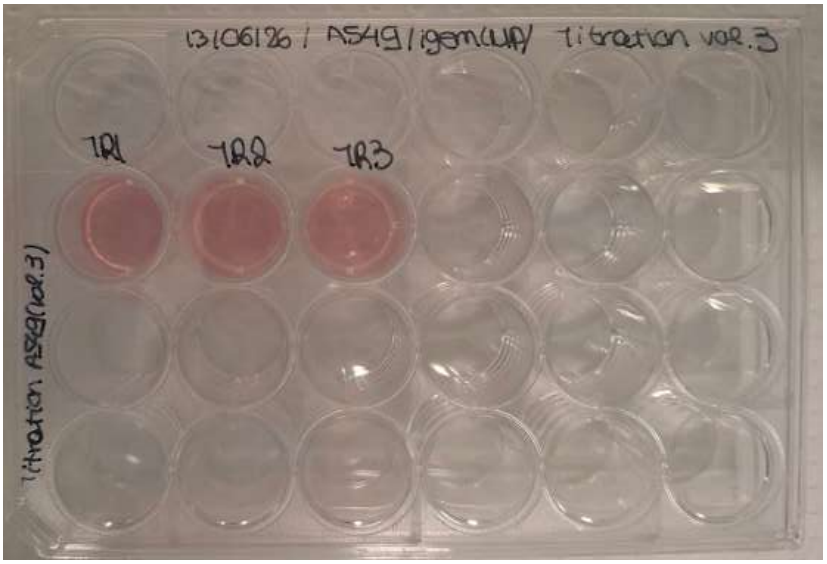
- **Date** – Sunday, June 14th 2026
- **Time (Start–End)** – 8:50 - 9:50
- **Name / Initials** – Margarita Papastergiou (MP)
- **Project / Subproject** – A-549 Cell Seeding Optimization: Cell Density Titration and Morphology Assessment Over 72 Hours (vol. 3)
- **Experiment / Task Title** – Observation of cell density 24h post-seeding.
- **Objective** – The purpose of today's experiment was to observe the different cell densities of A-549 cells, 24 hours post-seeding.

Experimental documentation

- **Materials / Reagents Used**
  - ❖ Inverted microscope
- **Method / Protocol Reference**
  - ➢ Cell seeding protocol for A-549 cells - Titration edition
- **Sample / Cell Line**
  - ➢ cell line: A-549

Observations & Results - day 1

- The color of the medium had changed from magenta to a paler pink-orange, indicating that the cells had started to metabolise and that the pH of their environment was changing.
- The cells had mostly taken up the center of the wells, but were also present at the perimeter of the wells.
- The bubbles that were created the day prior had disappeared.
- The technical replicates showed consistent morphology and no major observable differences during microscopic assessment.



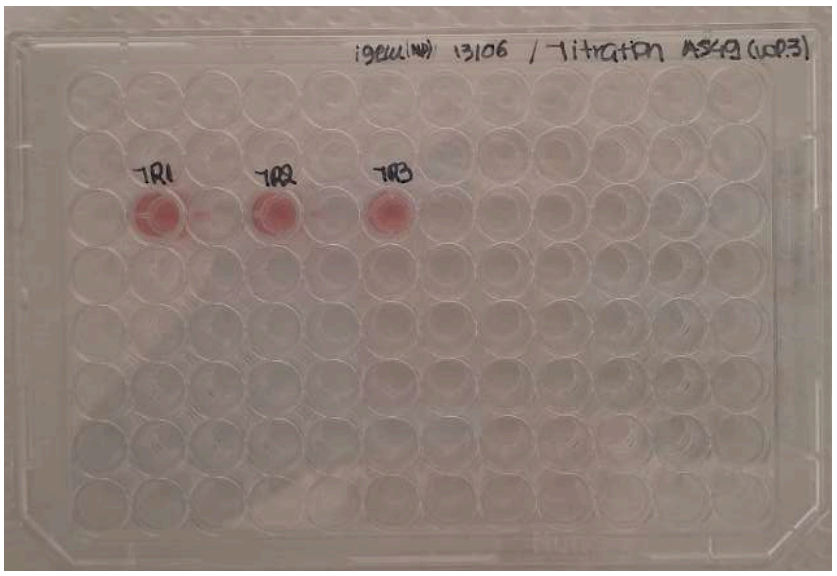


Figure x: Representative picture of the 24-well plate (up) and 96-well plate (down) 24 hours post cell-seeding.

JUNE 15TH 2026

Overview

- **Date** – Monday, June 14th 2026
- **Time (Start–End)** – 9:00 - 9:40
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Erriketti Moumka, Alexandros Vlachos
- **Project / Subproject** – A-549 Cell Seeding Optimization: Cell Density Titration and Morphology Assessment Over 72 Hours (vol. 3)
- **Experiment / Task Title** – Observation of cell density 48h post-seeding and reculture.
- **Objective** – The purpose of today's experiment was to observe the different cell densities of A-549 cells, 48 hours post-seeding, and change the medium of the cells.

Experimental documentation

- **Materials / Reagents Used**
  - ❖ Inverted microscope
  - ❖ stripettes 10 mL (x2)
  - ❖ DMEM FBS 10% 1% p/s (9 mL)
  - ❖ 30 ml Falcon (x1)
- **Method / Protocol Reference**
  - ➢ Cell seeding protocol for A-549 cells - Titration edition
- **Sample / Cell Line**
  - ➢ cell line: A-549

Observations & Results - day 2

- The color of the medium had changed from magenta to a paler pink-orange, indicating that the cells had started to metabolise and that the pH of their environment was changing.
- The cells had mostly taken up the center of the wells, but were also present at the perimeter of the wells.
- The technical replicates showed consistent morphology and no major observable differences during microscopic assessment.

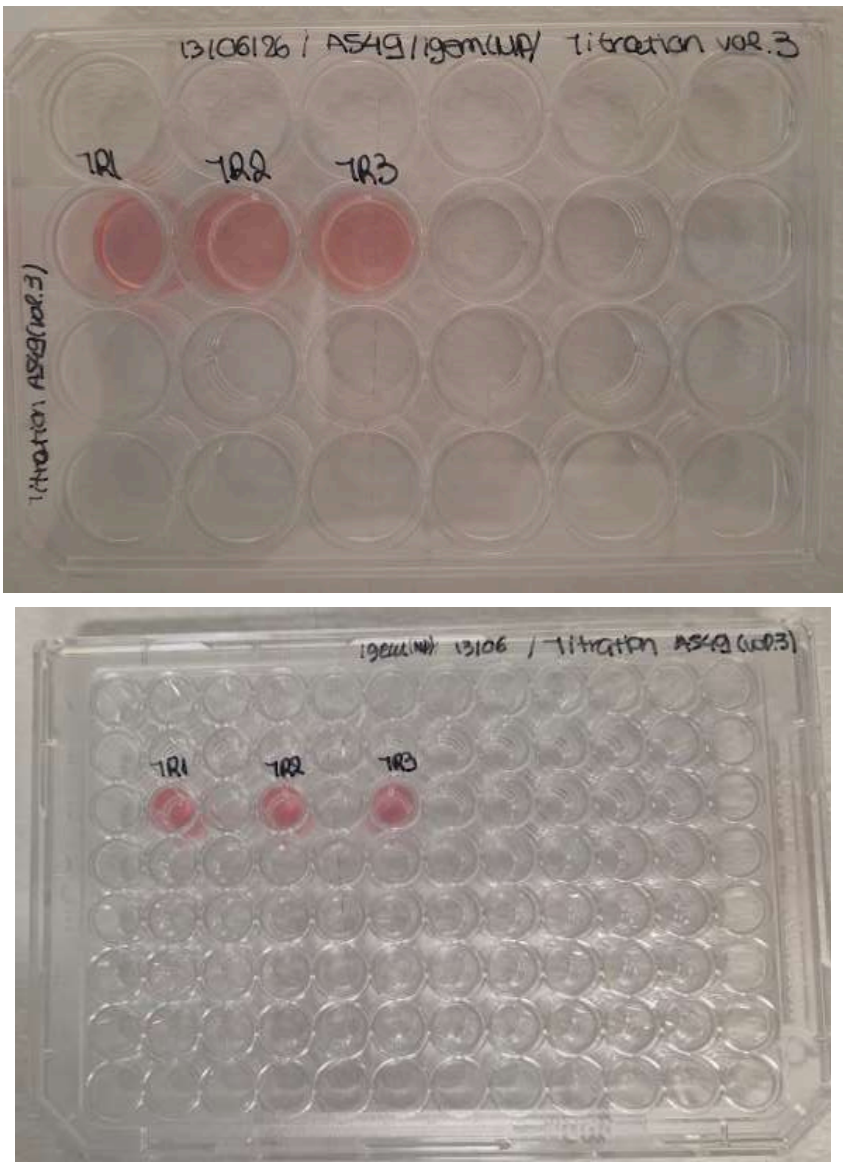


Figure x: Representative picture of the 24-well plate (up) and 96-well plate (down) 48 hours post cell-seeding.



Figure x: Representative image under microscope, A-549 cells in 5x focus (left) and 10x focus (right), before medium change.

JUNE 16TH 2026

Overview

- **Date** – Tuesday, June 16th 2026
- **Time (Start–End)** – 9:00 - 9:30
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Erriketti Moumka, Alexandros Vlachos
- **Project / Subproject** – A-549 Cell Seeding Optimization: Cell Density Titration and Morphology Assessment Over 72 Hours (vol. 3)
- **Experiment / Task Title** – Observation of cell density 72 h post-seeding and reculture.
- **Objective** – The purpose of today's experiment was to observe the different cell densities of A-549 cells, 72 hours post-seeding.

Experimental documentation

- **Materials / Reagents Used**
  - ❖ Inverted microscope
- **Method / Protocol Reference**
  - ➢ Cell seeding protocol for A-549 cells - Titration edition
- **Sample / Cell Line**
  - ➢ cell line: A-549

Observations & Results - day 3

- The color of the medium had changed from magenta to a paler pink-orange, indicating that the cells had started to metabolise and that the pH of their environment was changing.
- The cells had mostly taken up the center of the wells, but were also present at the perimeter of the wells.
- The technical replicates showed consistent morphology and no major observable differences during microscopic assessment.





Figure x: Representative picture of the 24-well plate (up) and 96-well plate (down) 72 hours post cell-seeding.

Conclusion of titration of cells/well (vol. 3)

- The cell density of **40,000 cells/well** provided a good initial seeding density for the **24-well plate**, resulting in sufficient cell attachment and well coverage. At **24 h post-seeding**, the cells covered **more than 60% of the well surface**, indicating that this density creates a suitable foundation for further experimental procedures.
- In contrast, the cell density of **25,000 cells/well** appeared to be too high for the **96-well plate format**. Due to the limited surface area and volume of the wells, several cells had not fully settled or attached to the bottom of the well and were observed at different focal planes, suggesting overcrowding or insufficient space for uniform distribution.
- After **48 h**, cell number and well coverage increased further, consistent with the expected doubling time of the cells. As a result, the confluency/coverage became higher compared to the 24 h observation.

JUNE 17TH 2026

Overview

- **Date** – Wednesday, June 16th 2026
- **Time (Start–End)** – 9:00 - 9:20 (reculture) / 11:10-12:10 (pellets)
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Erriketti Moumka, Alexandros Vlachos
- **Project / Subproject** – A-549 Cell Seeding Optimization: Cell Density Titration and Morphology Assessment Over 72 Hours (vol. 3)
- **Experiment / Task Title** – Pellets of cells from the 3 different TRs
- **Objective** – The purpose of today's experiment was to pellet the cells from the 3 TRs of the 24 and 96-well plates, combining the same TRs of each plate, resulting in 3 total tubes. Also, the medium was changed for the cells.

Experimental documentation

- **Materials / Reagents Used** **(link to consumables form)**
  - ❖ Inverted microscope
  - ❖ stripettes 10 mL (x2)
  - ❖ DMEM FBS 10% 1% p/s (11,1 mL)
  - ❖ 30 ml Falcon (x2)
  - ❖ Trypsin-EDTA (0.05%) (1,05 mL)
  - ❖ 1 mL tips (x12)
  - ❖ 200 µL tips (x12)
  - ❖ 20 µL tips (x6)
  - ❖ 1,5 mL tubes (x9)
- **Method / Protocol Reference**
  - ➢ Cell seeding protocol for A-549 cells - Titration edition
- **Sample / Cell Line**
  - ➢ cell line: A-549

Observations & Results

- The color of the medium had changed from magenta to a paler pink-orange, indicating that the cells had started to metabolise and that the pH of their environment was changing.
- The cells had taken up around 50% of the surface of the T-75 flask.
- After the trypsin step, no cells were left behind in the wells.
- The tubes containing the pellets are stored in the blue box at the molecular laboratory of the Pharmacology building.



Figure x: Representative image under microscope, A-549 cells in 5x focus (left) and 10x focus (right), before medium change.

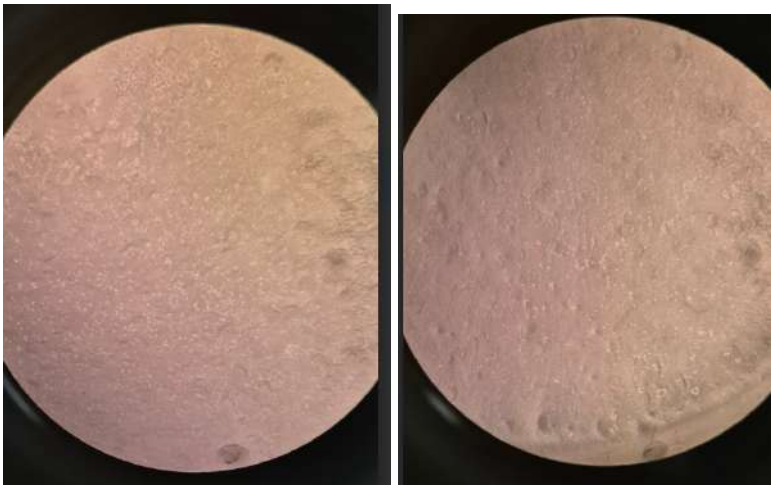


Figure x: Representative image under microscope, 24-well plate (left) and 96-well plate (right), after transferring all the cells to their corresponding tubes.

JUNE 19TH 2026

Overview

- **Date** – Friday, June 19th 2026
- **Time (Start–End)** – 8:02 - 9:43
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Erriketti Moumka, Alexandros Vlachos
- **Project / Subproject** – A-549 Cell Seeding Optimization: Cell Density Titration and Morphology Assessment Over 72 Hours [vol. 4]
- **Experiment / Task Title** – Medium change for cells and cell seeding for two conditions with 3 technical replicates each (10k and 12,5k cells/well).
- **Objective** – The purpose of today's experiment was to check the T-75 flask and change the medium, as well as perform the cell seeding for the titration of cells/well in a 96-well plate.

Experimental documentation

- **Materials / Reagents Used**
  - ➢ stripettes 10 mL (x5)
  - ➢ stripettes 5 mL (x3)
  - ➢ DMEM FBS 10% 1% p/s (15 mL)
  - ➢ 15 ml Falcon (x1)
  - ➢ 96-well plate (x1)
  - ➢ 1.5 ml tubes (x5)
  - ➢ PBS (3 ml)
  - ➢ Trypsin - EDTA (0.05%) (1,5 ml)
  - ➢ Trypan Blue (30 µL)
  - ➢ 10 µL tips (x6)
  - ➢ 1000 µL tips (x8)
- **Method / Protocol Reference**
  - ➢ In-house protocol for changing the medium
  - ➢ Cell seeding protocol for A-549 cells - Titration edition
- **Sample / Cell Line**
  - ➢ cell line: A-549

Observations & Results - day 0:

Before cell seeding:

- The color of the medium had changed from magenta to a paler pink-orange, indicating that the cells had started to metabolise and that the pH of their environment was changing.
- The surface the cells had taken up was around 70%.



Figure x: Representative image under microscope, A-549 cells in 5x focus (left) and 10x focus (right), before medium change.

During cell seeding:

- After thorough pipetting up and down to homogenize the cell suspension, an aliquot of 1 mL was taken from the flask, from which 20 µL were taken for Trypan Blue staining and cell counting using a Neubauer chamber.
- After the cell counting process and calculations, the concentration in the flask was 62.500 cells/mL.
- The volumes taken from the flask were: 2.08 mL for the 3 TRs of the 96-well plate for 10k cells/well and 1.67 mL for the 3 TRs of 12.5 k cells/well.
- The corresponding volumes for each condition were collected from the T-75 flask and centrifuged at 1200 rpm for 5 min. After the centrifuge step, pellets were present in all tubes.

After cell seeding:

- The wells were observed, and cells were present.
- No abnormalities were noted.

JUNE 20TH 2026

Overview

- **Date** – Saturday, June 20th 2026
- **Time (Start–End)** – 8:50 - 9:30
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Erriketti Moumka, Alexandros Vlachos
- **Project / Subproject** – A-549 Cell Seeding Optimization: Cell Density Titration and Morphology Assessment Over 72 Hours (vol. 4)
- **Experiment / Task Title** – Observation of cell density 24h post-seeding.
- **Objective** – The purpose of today's experiment was to observe the different cell densities of A-549 cells, 24 hours post-seeding.

Experimental documentation

- **Materials / Reagents Used**
  - ❖ Inverted microscope
- **Method / Protocol Reference**
  - ➢ Cell seeding protocol for A-549 cells - Titration edition
- **Sample / Cell Line**
  - ➢ cell line: A-549

Observations & Results - day 1

- The color of the medium had changed from magenta to a paler pink-orange, indicating that the cells had started to metabolise and that the pH of their environment was changing.
- The cells had mostly taken up the center of the wells, but were also present at the perimeter of the wells.
- The technical replicates showed consistent morphology and no major observable differences during microscopic assessment.

JUNE 20TH 2026

Overview

- **Date** – Thursday, June 20th 2026
- **Time (Start–End)** – 9:00 - 9:45
- **Name / Initials** – Margarita Papastergiou (MP), Erriketti Moumka (EM)
- **Shadowing** - Alexandros Vlachos
- **Project / Subproject** – A-549 Cell Seeding Optimization: Cell Density Titration and Morphology Assessment Over 72 Hours [vol.4]
- **Experiment / Task Title** – Observation of cell density 48 h post-seeding [vol.4].
- **Objective** – The purpose of today's experiment was to observe the different cell densities of A-549 cells, 48 hours post-seeding, and reculture (EM).

Experimental documentation

- **Materials / Reagents Used**
  - ❖ Inverted microscope
  - ❖ stripettes 10 mL (x2)
  - ❖ DMEM FBS 10% 1% p/s (9 mL)
  - ❖ 30 ml Falcon (x1)
- **Method / Protocol Reference**
  - ➢ In-house protocol for changing the medium
  - ➢ Cell seeding protocol for A-549 cells
- **Sample / Cell Line**
  - ➢ cell line: A-549

Observations & Results: Day 2

- The color of the medium had changed from magenta to a paler pink-orange, indicating that the cells had started to metabolise and that the pH of their environment was changing.
- The cells appeared to be in higher confluence relative to the previous days after seeding, in accordance with the cells' observed doubling time. This indicated that the environment and tested seeding densities were favorable to cell growth and division.
- The technical replicates showed consistent morphology and no major observable differences during microscopic assessment.

Reculture

Observation

- The color of the medium had changed from magenta to a paler pink-orange, indicating that the cells had started to metabolise and that the pH of their environment was changing. Culture volume had changed from 10ml to 9.8 ml of medium.
- The surface the cells had taken up was around 35-40%

Results

- The cells were observed, and no abnormalities were noted
- The cells were recultured in 10 ml of growth medium



Figure x: Representative image under microscope, A-549 cells in 5x focus (left) and 10x focus (right), before medium change.

JUNE 22ND 2026

Overview

- **Date** – Monday, June 22nd 2026
- **Time (Start–End)** – 8:30 - 9:00
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Alexandros Vlachos, Erriketti Moumka
- **Project / Subproject** – A-549 Cell Seeding Optimization: Cell Density Titration and Morphology Assessment Over 72 Hours [vol.4]
- **Experiment / Task Title** – Observation of cell density 72 h post-seeding [vol.4].
- **Objective** – The purpose of today's experiment was to observe the different cell densities of A-549 cells, 72 hours post-seeding, and make one pellet, containing all cells from the 2 different conditions observed.

Experimental documentation

- **Materials / Reagents Used**
  - ❖ Inverted microscope
  - ❖ DMEM FBS 10% 1% p/s (0,6 mL)
  - ❖ Trypsin-EDTA (0,05%) (0,3 mL)
  - ❖ 200 μL tipes (x13)
  - ❖ PBS 1x (0,3 mL)
  - ❖ 15 ml Falcon (x1)
  - ❖ 1,5 mL tube (x1)
- **Method / Protocol Reference**
  - ➢ In-house protocol for changing the medium
  - ➢ Cell seeding protocol for A-549 cells
- **Sample / Cell Line**
  - ➢ cell line: A-549

Observations & Results: Day 3

- The color of the medium had changed from magenta to a paler pink-orange, indicating that the cells had started to metabolise and that the pH of their environment was changing.
- The cells appeared to be in higher confluence relative to the previous days after seeding, in accordance with the cells' observed doubling time. This indicated that the environment and tested seeding densities were favorable to cell growth and division.
- The technical replicates showed consistent morphology and no major observable differences during microscopic assessment.

JUNE 23RD 2026

Overview

- **Date** – Tuesday, June 23rd 2026
- **Time (Start–End)** – 9:00 - 9:20
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Erriketti Moumka, Alexandros Vlachos
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Medium change for cells.
- **Objective** – The purpose of today's experiment was to check the T-75 flask and change the medium.

Experimental documentation

- **Materials / Reagents Used**
  - ➢ stripettes 10 mL (x2)
  - ➢ DMEM FBS 10% 1% p/s (9 mL)
  - ➢ 15 ml Falcon (x1)
- **Method / Protocol Reference**
  - ➢ In-house protocol for changing the medium
- **Sample / Cell Line**
  - ➢ cell line: A-549

Observations & Results

- **Observations / Notes**
  - The color of the medium had changed from magenta to a paler pink-orange, indicating that the cells had started to metabolise and that the pH of their environment was changing.
  - The surface the cells had taken up was around 40%.

Result Summary

- The cells were observed, and no abnormalities were noted.

Pictures in drive: link



Figure x: Representative image under microscope, A-549 cells in 5x focus (left) and 10x focus (right), before medium change.

JUNE 25TH 2026

Overview

- **Date** – Thursday, June 25th 2026
- **Time (Start–End)** – 9:00 - 12:38
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Erriketti Moumka, Alexandros Vlachos
- **Project / Subproject** – Cellular effect of Nutlin-3a in A-549 cell line (day 0/4)
- **Experiment / Task Title** – Seeding of cells for Nutlin-3a treatment and reculture of A-549 cells.
- **Objective** – The purpose of today's experiment was to seed A-549 cells in a 96-well plate for the Nutlin-3a treatment that was planned for the following days.

Experimental documentation

- **Materials / Reagents Used**
  - ➢ stripettes 10 mL (x5)
  - ➢ 96-well plate (x1)
  - ➢ stripettes 5 mL (x5)
  - ➢ DMEM FBS 10% 1% p/s (16,2 mL)
  - ➢ Trypsin - EDTA (0.005%) (1,5 mL)
  - ➢ PBS 1x (3 mL)
  - ➢ 30 mL Falcon (x1)
  - ➢ 50 mL Falcon (x2)
  - ➢ 1,5 mL tubes (x5)
  - ➢ filter (x1)
  - ➢ Syringe 10 mL (x1)
  - ➢ 1000 µL tips (x5)
  - ➢ 200 µL tips (x20)
  - ➢ 20 µL tips (x6)
  - ➢ Trypan Blue (30 µL)
  - ➢ FBS (5 mL)
  - ➢ DMEM (44,5 mL)
  - ➢ Pen/Strep (0,5 mL)
- **Method / Protocol Reference**
  - ➢ In-house protocol for changing the medium
  - ➢ Cellular effect of Nutlin-3a in the A-549 cell line protocol
- **Sample / Cell Line**
  - ➢ Cell line: A-549

Observations & Results

- **Pre-seeding**
  - The color of the medium had changed from magenta to a paler pink-orange, indicating that the cells had started to metabolise and that the pH of their environment was changing.

    - The surface the cells had taken up was around 80%.
    - Each well would have 12.500 cells/well => each timepoint would have 6 different conditions in 2 TRs => 12 wells/timepoint => 12*12.500 = 150.000 cells.
- **During seeding**
    - After the centrifuging step, pellets were present in all 3 tubes.
    - The pellet was resuspended in full medium, and 200 µL was added to each well.
    - During the seeding process, some bubbles were created in a few wells.

Result Summary

- The cells were observed, and no abnormalities were noted.
- All technical replicates showed similar morphology and cell density.

JUNE 26TH 2026

Overview

- **Date** – Friday, June 26th 2026
- **Time (Start–End)** – 8:30 - 11:10
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Erriketti Moumka, Alexandros Vlachos
- **Project / Subproject** – Cellular effect of Nutlin-3a in A-549 cell line (day 1/4)
- **Experiment / Task Title** – Treatment of A-549 cells with Nutlin-3a.
- **Objective** – The purpose of today's experiment was to treat A-549 cells with Nutlin-3a at different concentrations.

Experimental documentation

- **Materials / Reagents Used**
  - ➢ DMEM FBS 10% 1% p/s (9,6 mL)
  - ➢ 30 ml Falcon (x1)
  - ➢ 200 µL tips (x20)
  - ➢ 1,5 mL tube (x1)
  - ➢ 10 µL tips (x16)
  - ➢ 1000 µL tips (x12)
  - ➢ DMSO (21,2 µL)
  - ➢ 15 mL Falcon (x1)
  - ➢ Nutlin-3a stock 10 mM (16,2 µL)
  - ➢ 10 mL stripettes (x2)
  - ➢ 5 mL stripettes (x1)
  - ➢ filter (x2)
  - ➢ 50 mL Falcons (x2)
  - ➢ FBS (10 mL)
  - ➢ Pen/strep (1 mL)
  - ➢ DMEM (89 mL)
- **Method / Protocol Reference**
  - ➢ In-house protocol for changing the medium
  - ➢ Cellular effect of Nutlin-3a in the A-549 cell line protocol
- **Sample / Cell Line**
  - ➢ cell line: A-549

Observations & Results

- **Pre-treatment**
  - The wells were observed, and more than 60% of the surface was covered, allowing us to proceed with the treatment.
  - A stock of 2 mM Nutlin-3a was prepared to simplify pipetting and master mix preparation.
  - Each master mix was comprised of: Nutlin-3a, DMSO, and full medium.
  - The master mixes were prepared based on the following matrix:

Condition	Nutlin-3a 10 mM stock (µL)	Nutlin-3a 2 mM stock (µL)	DMSO (µL)	Full medium (µL)
0 µM	0	0	6,4	1593,6
2,5 µM	0	2	4,4	1593,6
5 µM	0	4	2,4	1593,6
10 µM	1,6	0	4,8	1593,6
20 µM	3,2	0	3,2	1593,6
40 µM	6,4	0	0	1593,6

    - The final volume of each mastermix was 1600 µL (1400 µL for all timepoints/condition and 200 µL buffer zone for any potential pipetting errors)

*The 2 mM N-3a stock was created from the left N-3a 10 mM stock (the one with the least volume) -> left on the ice cube
**For the 20 and 40 µM mastermix, the 2nd 10 mM N-3a stock was used (the one with the larger volume) -> right on the ice cube
***For the 10 µM mastermix, the left N-3a 10 mM stock (the one with the least volume) -> left on the ice cube

- **During the treatment**
    - The final volume of each well was 200 µL. The treatment was shared per condition across all timepoints, in two intervals: 100 µL from the 0 µM condition to the 40 µM condition, and 100 µL from the 40 µM condition to the 0 µM condition.
    - After the treatment, every mastermix contained 400 µL.

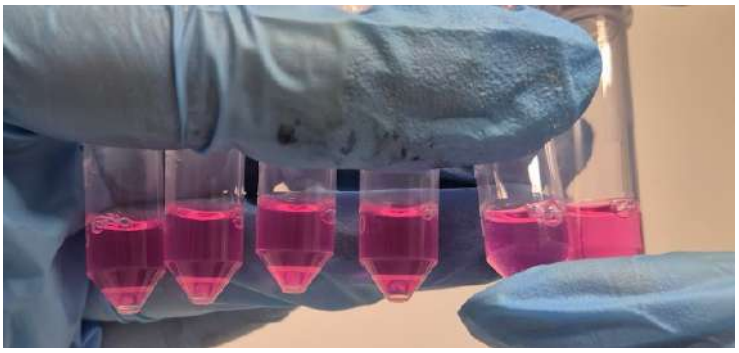




Figure X: The remaining volume of each mastermix, across all conditions.

- **Post-treatment**
    - The cells were observed, and no abnormalities were noted.

JUNE 27TH 2026

Overview

- **Date** – Saturday, June 27th 2026
- **Time (Start–End)** – 9:00 - 13:30
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Erriketti Moumka
- **Project / Subproject** – Cellular effect of Nutlin-3a in A-549 cell line (day 2/4)
- **Experiment / Task Title** – Cell viability assay of A-549 cells with Nutlin-3a, 24 h post-treatment and reculture.
- **Objective** – The purpose of today's experiment was to check the cell viability of A-549 cells treated with Nutlin-3a at different concentrations, 24 hours post-treatment.

Experimental documentation

- **Materials / Reagents Used**
  - ➢ DMEM FBS 10% 1% p/s (10,8 mL)
  - ➢ 30 mL Falcon (x1)
  - ➢ 200 µL tips (x40)
  - ➢ 1,5 mL tube (x42)
  - ➢ 10 µL tips (x54)
  - ➢ 1000 µL tips (x6)
  - ➢ Trypan blue (180 µL)
  - ➢ Trypsin (600 µL)
  - ➢ PBS 1x (600 µL)
  - ➢ 10 mL stripettes (x2)
- **Method / Protocol Reference**
  - ➢ In-house protocol for changing the medium
  - ➢ Cellular effect of Nutlin-3a in the A-549 cell line protocol
- **Sample / Cell Line**
  - ➢ Cell line: A-549

Observations & Results

- The color of the medium had changed from magenta to a paler pink-orange, indicating that the cells had started to metabolise and that the pH of their environment was changing, in the 96-well plate.
- The cells were washed with PBS, and trypsin was added to detach them from their surface. Medium was added to deactivate the trypsin, and the volume of each well was divided into 2 tubes: half for the TR and half for the pool of the 2 TRs.
- After the collection of the volume was completed, 20 µL of each tube was taken for Trypan blue staining. The rest was combined into one tube and was used to make pellets.
- The pellets were stored at -80oC at the pharmacology building inside the blue box.

JUNE 28TH 2026

Overview

- **Date** – Sunday, June 28th 2026
- **Time (Start–End)** – 8:45 - 12:38
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Erriketti Moumka
- **Project / Subproject** – Cellular effect of Nutlin-3a in A-549 cell line (day 3/4)
- **Experiment / Task Title** – Cell viability assay of A-549 cells with Nutlin-3a, 48 h post-treatment.
- **Objective** – The purpose of today's experiment was to check the cell viability of A-549 cells treated with Nutlin-3a at different concentrations, 48 hours post-treatment.

Experimental documentation

- **Materials / Reagents Used**
  - ➢ DMEM FBS 10% 1% p/s (1,8 mL)
  - ➢ 30 mL Falcon (x1)
  - ➢ 200 µL tips (x40)
  - ➢ 1,5 mL tube (x42)
  - ➢ 10 µL tips (x54)
  - ➢ 1000 µL tips (x6)
  - ➢ Trypan blue (180 µL)
  - ➢ Trypsin (600 µL)
  - ➢ PBS 1x (600 µL)
- **Method / Protocol Reference**
  - ➢ In-house protocol for changing the medium
  - ➢ Cellular effect of Nutlin-3a in the A-549 cell line protocol
- **Sample / Cell Line**
  - ➢ Cell line: A-549

Observations & Results

- The color of the medium had changed from magenta to a paler pink-orange, indicating that the cells had started to metabolise and that the pH of their environment was changing, in the 96-well plate.
- The cells were washed with PBS, and trypsin was added to detach them from their surface. Medium was added to deactivate the trypsin, and the volume of each well was divided into 2 tubes: half for the TR and half for the pool of the 2 TRs.
- After the collection of the volume was completed, 20 µL of each tube was taken for Trypan blue staining. The rest was combined into one tube and was used to make pellets.
- The pellets were stored at -80oC at the pharmacology building inside the blue box.

JUNE 29TH 2026

Overview

- **Date** – Monday, June 27th 2026
- **Time (Start–End)** – 8:22 - 11:30
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Erriketti Moumka
- **Project / Subproject** – Cellular effect of Nutlin-3a in A-549 cell line (day 4/4)
- **Experiment / Task Title** – Cell viability assay of A-549 cells with Nutlin-3a, 72 h post-treatment and reculture.
- **Objective** – The purpose of today's experiment was to check the cell viability of A-549 cells treated with Nutlin-3a at different concentrations, 72 hours post-treatment.

Experimental documentation

- **Materials / Reagents Used**
  - ➢ DMEM FBS 10% 1% p/s (10,8 mL)
  - ➢ 30 mL Falcon (x1)
  - ➢ 200 µL tips (x40)
  - ➢ 1,5 mL tube (x42)
  - ➢ 10 µL tips (x54)
  - ➢ 1000 µL tips (x6)
  - ➢ Trypan blue (180 µL)
  - ➢ Trypsin (600 µL)
  - ➢ PBS 1x (600 µL)
  - ➢ 5 mL stripettes (x3)
- **Method / Protocol Reference**
  - ➢ In-house protocol for changing the medium
  - ➢ Cellular effect of Nutlin-3a in the A-549 cell line protocol
- **Sample / Cell Line**
  - ➢ Cell line: A-549

Observations & Results

- The color of the medium in the 96-well plate had changed from magenta to a paler pink-orange, indicating that the cells had started to metabolise and that the pH of their environment was changing.
- The cells were washed with PBS, and trypsin was added to detach them from their surface. Medium was added to deactivate the trypsin, and the volume of each well was divided into 2 tubes: half for the TR and half for the pool of the 2 TRs.
- After the collection of the volume was completed, 20 µL of each well was taken for Trypan blue staining. The rest was combined into one tube and was used to make pellets.

- The pellets were stored at -80oC at the pharmacology building inside the blue box.

JULY 14TH 2026

Overview

- **Date** – Tuesday, July 14th 2026
- **Time (Start–End)** – 13:00 - 13:35
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Erriketti Moumka, Taxiarchis Kosmas, Anna Filindri, Ilias Theocharous
- **Project / Subproject** – Start of A-549 cell line (2nd opening)
- **Experiment / Task Title** – Preparation of medium.
- **Objective** – The purpose of today's experiment was to prepare medium for the upcoming cell thawing.

Experimental documentation

- **Materials / Reagents Used**
  - ➢ 50 mL Falcon (x2)
  - ➢ 1000 µL tips (x1)
  - ➢ 5 mL stripettes (x1)
  - ➢ 10 mL stripettes (x3)
  - ➢ FBS (5 mL)
  - ➢ Pen/Strep (0,5 mL)
  - ➢ DMEM (44,5 mL)
  - ➢ filter
  - ➢ syringe
- **Method / Protocol Reference**
  - ➢ In-house protocol for medium preparation
- **Sample / Cell Line**
  - ➢ Cell line: A-549

Observations & Results

- The color of the reagents was okay, and no abnormalities were noted.

JULY 15TH 2026

Overview

- **Date** – Tuesday, July 15th 2026
- **Time (Start–End)** – 9:00 - 11:00
- **Name / Initials** – Margarita Papastergiou (MP), Erriketti Moumka (EM)
- **Shadowing** -Alexandros Vlachos
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Thawing of A-549 cell line
- **Objective** – The purpose of this day's experiment was to thaw the A-549 cell line to start the cell culture process.

Experimental documentation

- **Materials / Reagents Used**
  - ➢ T-25 flask (x1)
  - ➢ stripettes 5 mL (x7)
  - ➢ stripettes 10 mL (x3)
  - ➢ DMEM FBS 10% 1% p/s (6 mL)
  - ➢ 15 ml Falcon (x2)
  - ➢ 30 ml Falcon (x1)
  - ➢ 50 ml Falcon (x2)
  - ➢ FBS (7,5 mL)
  - ➢ DMEM (42 mL)
  - ➢ Pen/Strep (0,5 mL)
  - ➢ filter
  - ➢ syringe
  - ➢ 1000 µL tips (x1)
  - ➢ 1,5 mL tubes (x1)
- **Method / Protocol Reference**
  - ➢ In-house protocol for cell thawing
- **Sample / Cell Line**
  - ➢ cell line: A-549

Observations & Results

- **Observations / Notes**
  - The pellet of cells after the centrifuge step was present in both cryovials used, indicating that the thawing step was done correctly.
  - After resuspending the pellet, the flask containing cells was observed, and no abnormalities were noted.
- **Result Summary**
  - The cells were observed, and no abnormalities were noted.

JULY 16TH 2026

Overview

- **Date** – Thursday, July 16th 2026
- **Time (Start–End)** – 9:40 - 10:30
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** -Alexandros Vlachos, Erriketti Moumka, Ilias Theocharous
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Observation of A-549 cells
- **Objective** – The purpose of this day's experiment was to observe the A-549 cells to assess their morphology and decide whether or not they need a change of medium.

Experimental documentation

- **Materials / Reagents Used**

-

Observations & Results

- **Observations / Notes**
    - The color of the medium was changed to a paler pink, indicating that the cells had begun to metabolise.
    - Most of the cells had formed a monolayer, but some had not settled.
- **Result Summary**
    - After the observation, we decided not to change the medium of the flask.

JULY 17TH 2026

Overview

- **Date** –Friday, July 17th 2026
- **Time (Start–End)** – 9:10 - 9:40
- **Name / Initials** – Margarita Papastergiou
- **Shadowing** -Alexandros Vlachos, Melita Lampropoulou, Ilias Theocharous
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Observation of A-549 cells and reculture
- **Objective** – The purpose of this day's experiment was to observe the A-549 cells and perform a reculture.

Experimental documentation

- **Materials / Reagents Used**
  - ❖ DMEM FBS 15% 1% p/s (5,5 mL)
  - ❖ 30 mL Falcon (x1)
  - ❖ 5 mL stripettes (x2)

Observations & Results

- **Observations / Notes**
  - The color of the medium was changed to a paler pink, indicating that the cells had begun to metabolise. The volume of the flask was 5,9 mL.
  - Most of the cells had settled down, so we performed a medium change.

Result Summary

- After the medium change, no abnormalities and differences were noted.

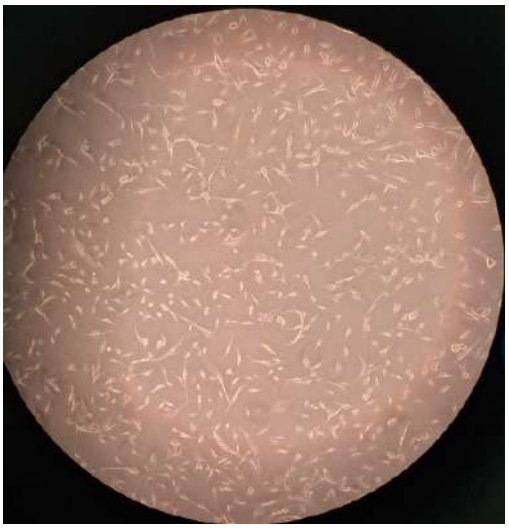


Figure x: Representative picture of flask before medium change

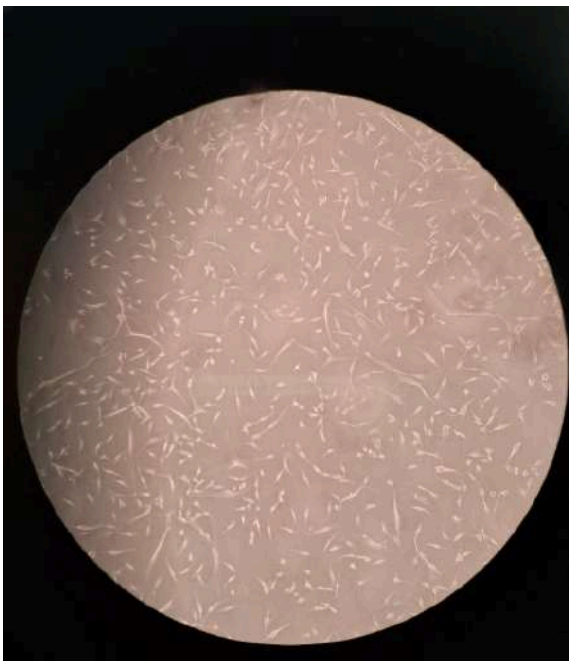


Figure x: Representative picture of flask after medium change

JULY 18TH 2026

Overview

- **Date** – Thursday, July 18th 2026
- **Time (Start–End)** – 9:40 - 10:40
- **Name / Initials** – Erriketti Moumka (EM)
- **Shadowing** - Alexandros Vlachos, Melita Lampropoulou, Anna Filindri
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Observation of A-549 cells, Reculture, Cryovial preservation
- **Objective** – The purpose of this day's experiment was to observe the A-549 cells to assess their morphology and decide whether or not they need a change of medium or if their confluency is ideal for cryopreservation in cryovials.

Experimental documentation

- **Materials / Reagents Used**

- DMEM FBS 15% 1% p/s (5,5 mL)
- 15 mL Falcon (×1)
- 5 mL stripettes (×2)

Observations & Results

- **Observations / Notes**
    - The color of the medium had changed to a paler pink-orange, indicating that the cells had begun to metabolise, and the residual medium had a volume of 5,8 ml.
    - The surface the cells had taken up was around 60-70% but there were still gaps visible with the ×10 microscope lens in places.
- **Result Summary**
    - After the observation, we decided that the cells needed one more day for the cryovial preparation
    - The cells were recultured in 6 mL total (0,5 mL old and 5,5 new) of complete medium supplemented with 15% FBS to boost their recovery following the thawing procedure.

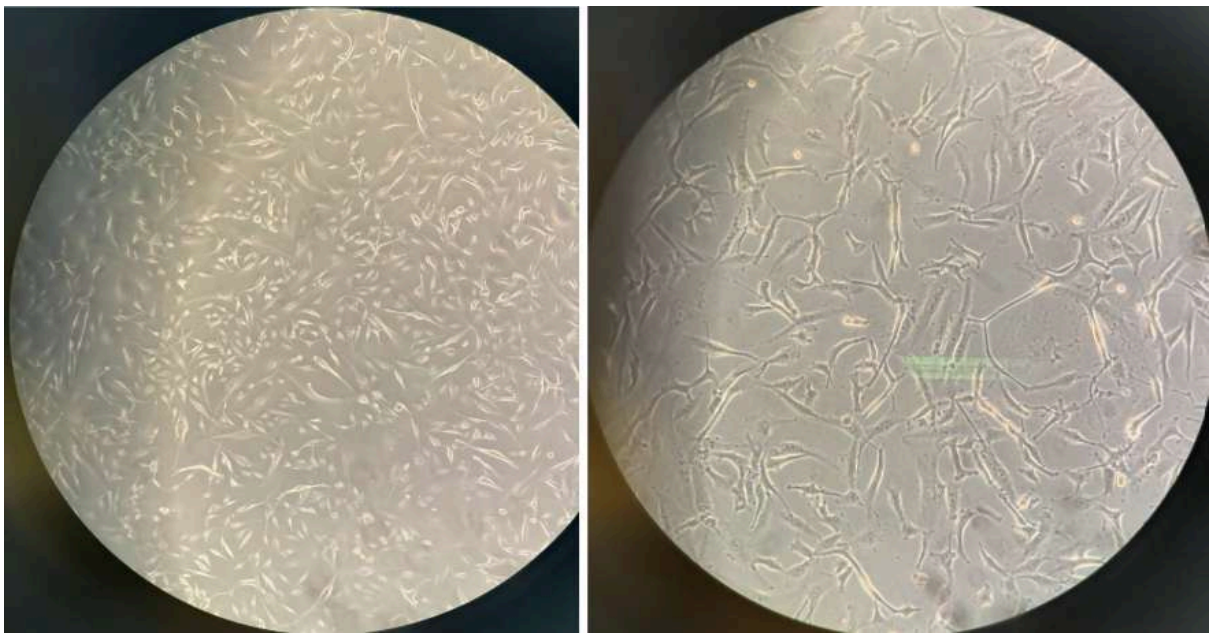


Figure x: Representative image under microscope, A-549 cells in 5x focus (left) and 10x focus (right), before medium change

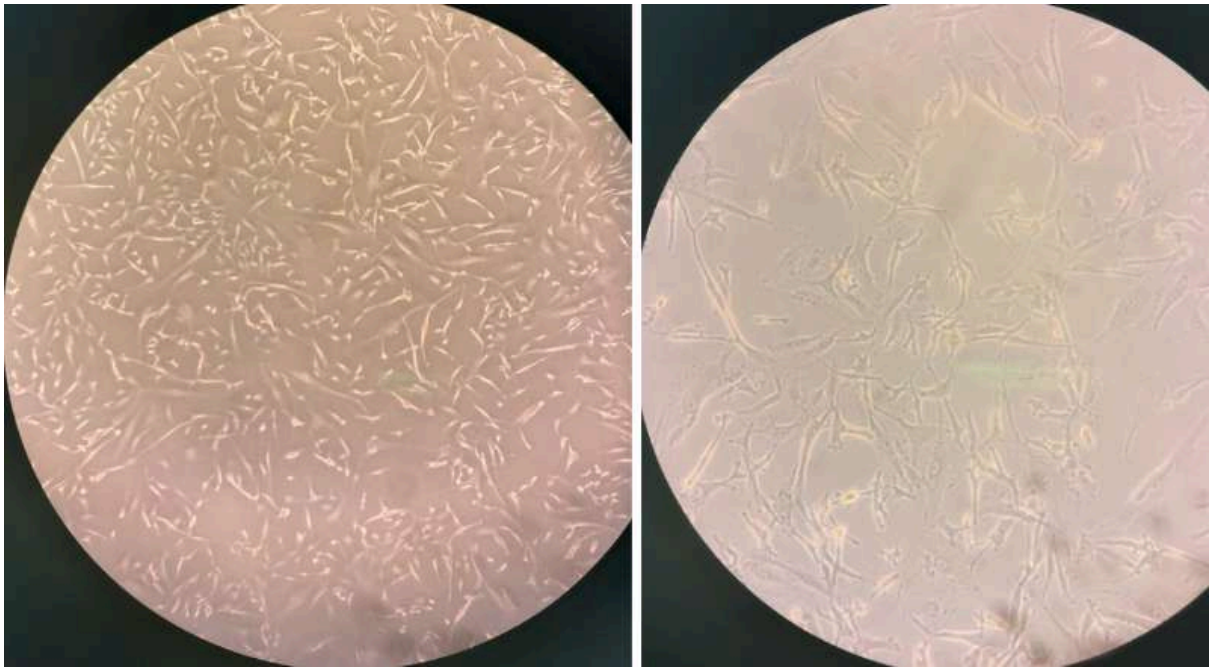


Figure x: Representative image under microscope, A-549 cells in 5x focus (left) and 10x focus (right), after medium change

JULY 19TH 2026

Overview

- **Date** – Sunday, July 19th 2026
- **Time (Start–End)** – 9:00- 11:50
- **Name / Initials** – Margarita Papastergiou, Erriketti Moumka
- **Shadowing** -Alexandros Vlachos, Anna Filindri
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Cryovials and reculture
- **Objective** – The purpose of this day's experiment was to make cryovials of the A-549 cell line and do a reculture.

Experimental documentation

- **Materials / Reagents Used**
  - DMEM FBS 15% 1% p/s (8 mL)
  - 30 mL Falcon (x1)
  - 1,5 mL tube (x2)
  - 10 µL tips (x10)
  - 1000 µL tips (x2)
  - Trypan blue (60 µL)
  - Trypsin (1 mL)
  - PBS 1x (1,5 mL)
  - 5 mL stripettes (x13)
  - 10 mL stripettes (x2)
  - 50 mL Falcon (x1)
  - 15 mL Falcons (x3)
  - DMSO (5 mL)
  - FBS (15 mL)
  - DMEM FBS 10% 1% p/s (30 mL)
  - cryovials with white cap (x2)

Observations & Results

- **Observations / Notes**
  - Fresh freezing medium was prepared prior to the procedure, as the freezing medium stored in the -20°C freezer was unsuccessfully located. We will check our inventory to pinpoint the issue.
  - The color of the medium had changed to a paler pink-orange, indicating that the cells had begun to metabolise, with a final volume of 6 mL.
  - The cells had taken up around 80% of the flask's surface.

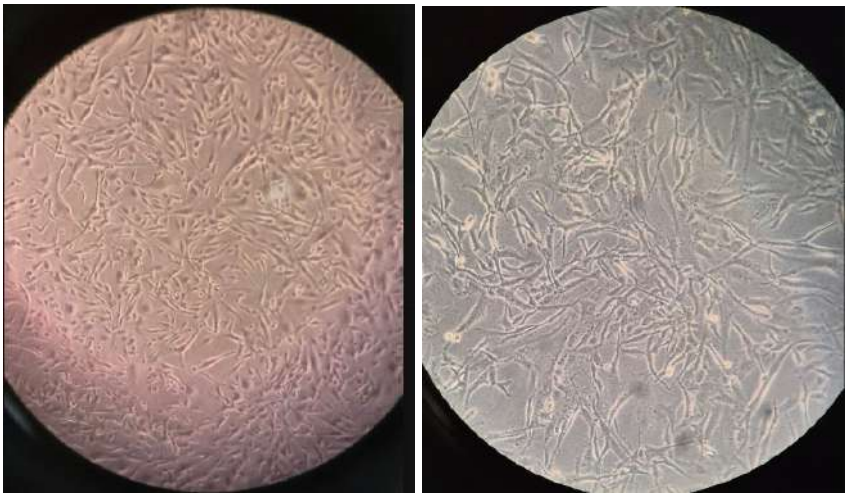


Figure x: Representative pictures of the T-25 flask before the addition of trypsin
Left: 5x focus, Right: 10x focus.

- 1mL of trypsin was added and it was deactivated by adding 2 mL of full medium => final volume: 3 mL
  - ⅓ of the flask's volume was used for the 1st cryovial (1 mL)
  - ⅓ of the flask's volume was used for the 2nd cryovial (1 mL)
  - ⅓ of the flask's volume was used for the reculture (1 mL)
    *the flask was observed and no cells were left behind.
- After centrifuging the Falcons containing the volumes above, a pellet was present at all 3 Falcons.
- The pellets of the first 2 Falcons were resuspended in 1 mL freezing medium, and the volume was transferred to the cryovials. The cryovials were immediately stored at -20 ℃ (freezer) for 20 minutes.
- After 20 minutes, the cryovials were stored at -80 ℃.
- Regarding the reculture procedure, the pellet was resuspended in 6 mL full medium, and the volume was transferred to the incubator (37 ℃, 5% CO2).

Trypan blue staining:

- During the cell counting step, we performed trypan blue staining at two different aliquots:
  - 1 aliquot of the old medium from the flask
  - 1 aliquot of the flask's volume after the deactivation of trypsin
    *both aliquots were taken after performing up and down thoroughly
- No cells were present in the Neubauer chamber from the aliquot containing the old medium from the flask.
- Cells were present in the Neubauer chamber from the aliquot containing volume from the flask after the deactivation of trypsin.

1st flask							
A549_T-25_MP	live	dead	Avg live	Avg dead	all	% Viability	cell/ml
5	13	2	14,25	1,5	15,75	90,47619048	**285000**
6	12	1					
7	16	2					
8	16	1					
						TOTAL C.N	855000

Result Summary

- After the observation, we decided that the cells were ready for the cryopreservation procedure.
- The cryopreservation procedure was done within the safe-for-the-cells timeframe.
- The cryovials are stored at -80 ℃ in the yellow box in the molecular lab.

JULY 20TH 2026

Overview

- **Date** – Monday, July 20th 2026
- **Time (Start–End)** – 9:00 - 9:35
- **Name / Initials** – Erriketti Moumka (EM)
- **Shadowing** - Margarita Papastergiou, Alexandros Vlachos, Taxiarchis Kosmas
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Observation of A-549 cells
- **Objective** – The purpose of this day's experiment was to observe the A-549 cells to assess their morphology and decide whether or not they need a change of medium.

Experimental documentation

- **Materials / Reagents Used**

-

Observations & Results

- **Observations / Notes**
    - The color of the medium was changed to a paler pink, indicating that the cells had begun to metabolise.
    - The surface the cells had taken up was around 40%
    - When observing the flask under the optical microscope, we observed different morphological characteristics that we had not observed before at such a widespread scale. With the 5x lens, spherical or square cells with what appeared like microscopic bubbles were observed across the surface of the flask, at the same level as the typical adherent cells. Under the 10x lens, some cells with otherwise typical shape appeared as if they had tiny holes.
- **Result Summary**
    - After the observation, we decided not to change the medium of the flask.
    - The flask was placed back in the incubator.
    - Our wet lab team was tasked with researching the unusual cell morphologies observed to rule out possible issues and better understand our cell line.

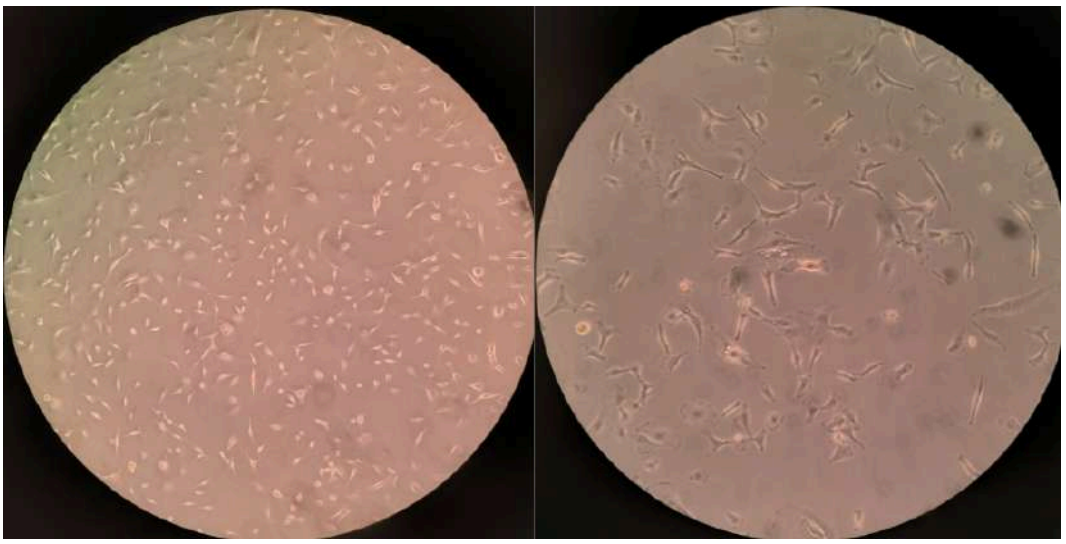


Figure x: Representative image under microscope, A-549 cells in 5x focus (left) and 10x focus (right)

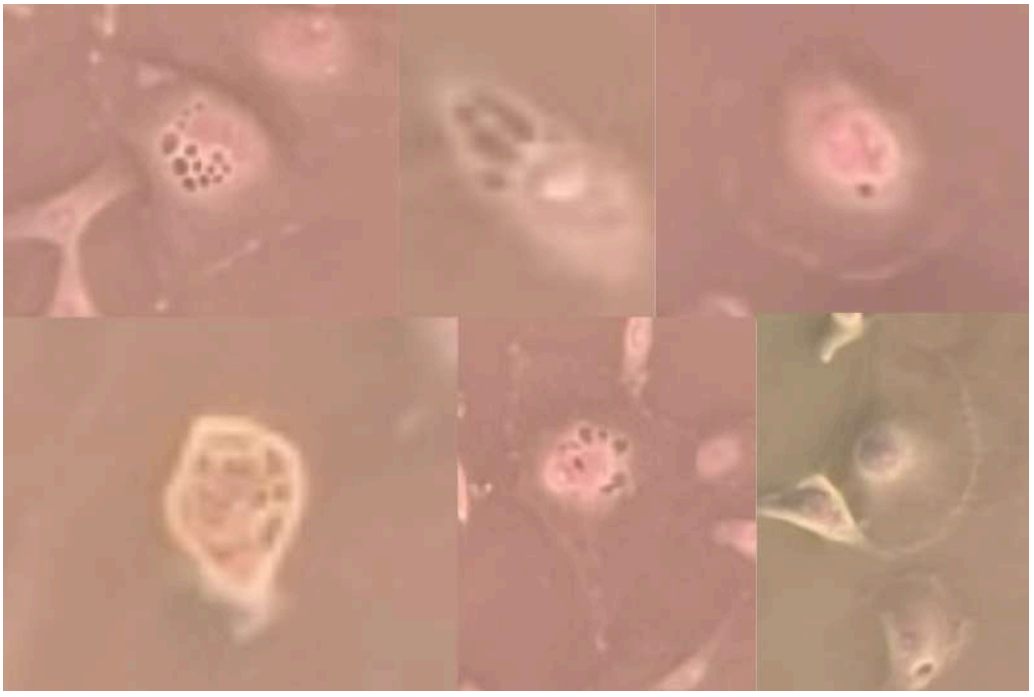


Figure x: A-549 cells in 5x focus. Magnification focusing on cells with unusual morphology

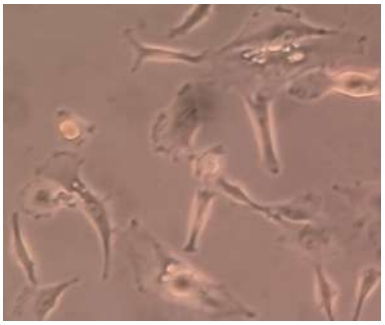


Figure x: A-549 cells in 10x focus. Magnification focusing on cells with unusual morphology

JULY 21ST 2026

Overview

- **Date** – Tuesday, July 21, 2026
- **Time (Start–End)** – 9:05 - 9:55
- **Name / Initials** – Erriketti Moumka (EM)
- **Shadowing** - Margarita Papastergiou, Alexandros Vlachos, Katerina
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Observation of A-549 cells and reculture
- **Objective** – The purpose of this day's experiment was to observe the A-549 cells and change the medium.

Experimental documentation

- **Materials / Reagents Used**
  - ➢ stripettes 5 mL (x2)
  - ➢ DMEM FBS 15% 1% p/s (5,5 mL)
  - ➢ 15 ml Falcon (x1)
- **Method / Protocol Reference**
  - ➢ In-house protocol for changing the medium
- **Sample / Cell Line**
  - ➢ cell line: A-549

Observations & Results

- **Observations / Notes**
  - The color of the medium had changed to a paler pink-orange, indicating that the cells had started to metabolise and that the pH of their environment was changing.
  - The surface the cells had taken up was around 50-60%.
  - When observing the flask under the microscope, the unusual cell morphologies observed yesterday were still present in similar low abundance. Some of the cells that were observed include round cells with contained bubbles and what seemed like a ring shape around them, while most of the unusual cells retained their shape but were signified by the apparent bubbles.
  - The residual medium had a volume of 5,7 mL.
- **Result Summary**
  - The cells were observed, and the abnormal findings were documented with representative photos.
  - The cells were recultured in 6 mL (5,5 mL new and 0,5 mL old) of complete growth medium with 15% FBS to boost their recovery.
  - Troubleshooting the morphological abnormalities continues, with the additional observation that this phenotype was still present 24h after initial observation.

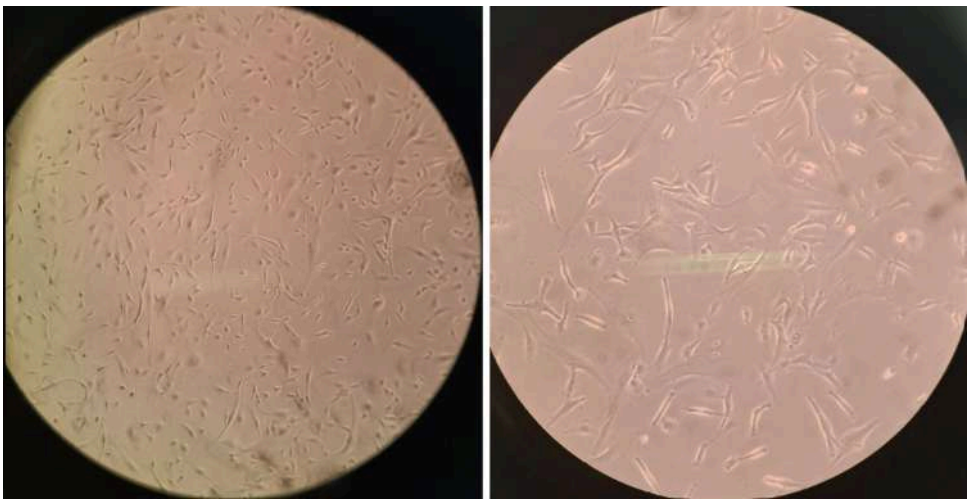


Figure x: Representative image under microscope before reculture, A-549 cells in 5x focus (left) and 10x focus (right)



Figure x: Representative image under microscope after reculture, A-549 cells in 5x focus (left) and 10x focus (right)

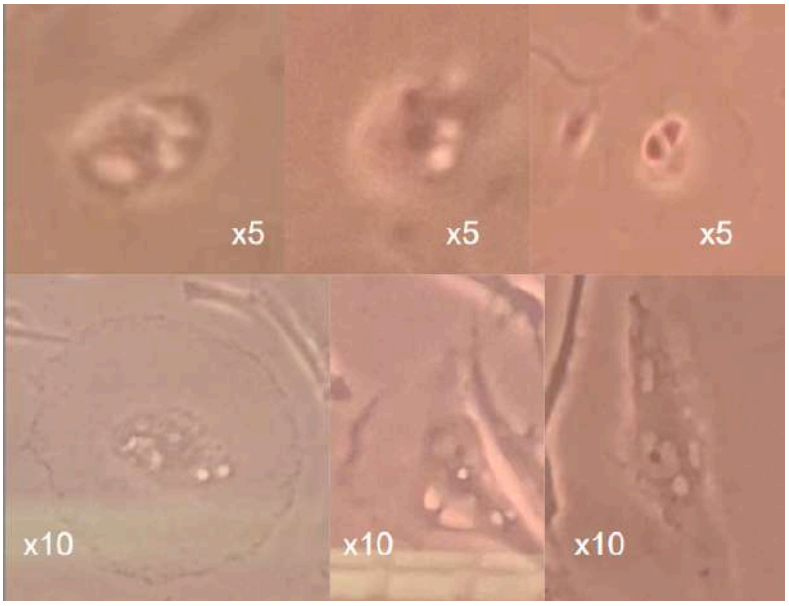


Figure x: Magnification focusing on cells with unusual morphology, A-549 cells in 5x focus (above) and 10x focus (below)

JULY 22TH 2026

Overview

- **Date** – Wednesday, July 22th 2026
- **Time (Start–End)** – 9:10 - 9:50
- **Name / Initials** – Erriketti Moumka (EM)
- **Shadowing** - Margarita Papastergiou, Alexandros Vlachos, Melita Lampropoulou
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Observation of A-549 cells
- **Objective** – The purpose of this day's experiment was to observe the A-549 cells to assess their morphology and decide whether or not they need a change of medium.

Experimental documentation

- **Materials / Reagents Used**

-

Observations & Results

- **Observations / Notes**
    - The color of the medium was changed to a paler pink, indicating that the cells had begun to metabolise.
    - The surface the cells had taken up was around 60-70%
    - When observing the flask under the microscope, the unusual cell morphologies observed the two previous days were still present in similar low abundance. Some of the cells that were observed include round cells with contained bubbles and what seemed like a ring shape around them, while most of the unusual cells retained their shape but were signified by the apparent bubbles.
- **Result Summary**
    - The cells were observed, and the abnormal findings were documented with representative photos.
    - After the observation, we decided not to change the medium of the flask, and instead reassess the cells' confluency tomorrow to potentially passage them to a T-75 flask.
    - The flask was placed back in the incubator.

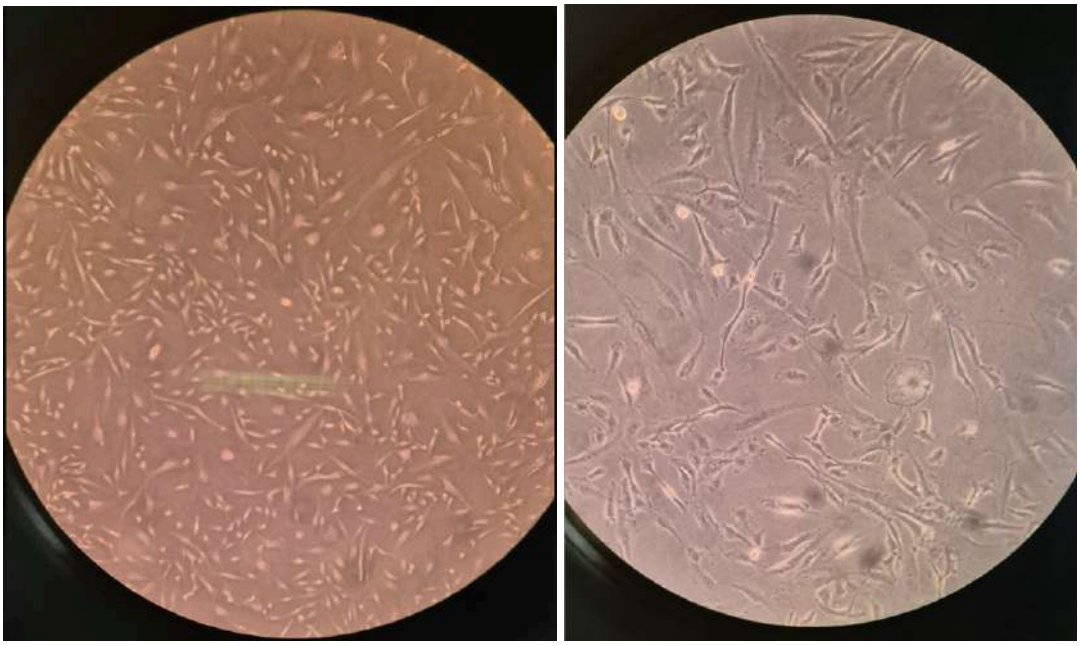


Figure x: Representative image under microscope, A-549 cells in 5x focus (left) and 10x focus (right)

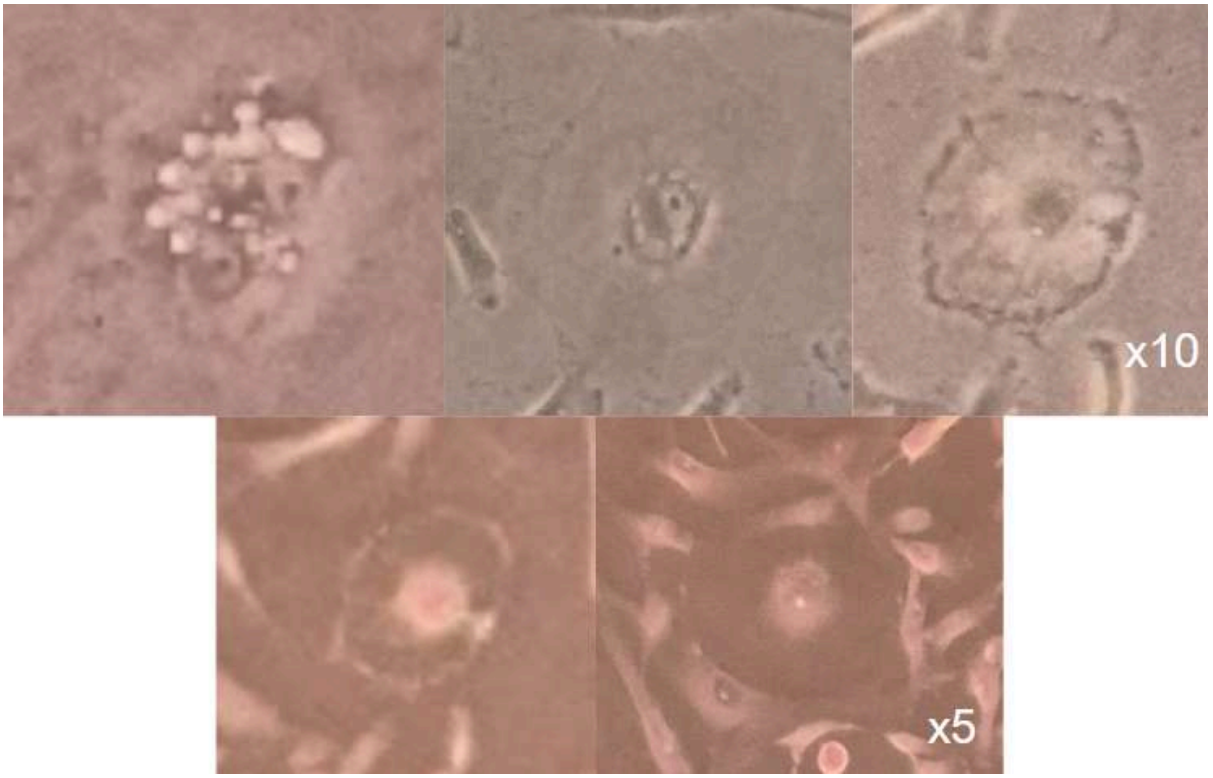


Figure x: Magnification focusing on cells with unusual morphology, A-549 cells in 10x focus (above) and 5x focus (below)

JULY 23RD 2026

Overview

- **Date** – Thursday, July 23, 2026
- **Time (Start–End)** – 9:10 - 12:45
- **Name / Initials** – Erriketti Moumka (EM)
- **Shadowing** - Margarita Papastergiou, Alexandros Vlachos, Anna Filindri
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Observation of A-549 cells, cell passaging, and media preparation
- **Objective** – The purpose of this day's experiment was to observe the A-549 cells and to passage a percentage of the cells to a new T-75 flask. Additionally, we prepared fresh complete media (MP). Finally, Alexandros Vlachos and Anna Filindri practiced using the Neubauer chamber to perform cell counting.

Experimental documentation

- **Materials / Reagents Used**
  - ➢ stripettes 5 mL (x7)
  - ➢ stripettes 10ml (x6)
  - ➢ 50 mL Falcon (x4)
  - ➢ 30 mL Falcon (x1)
  - ➢ 15 ml Falcon (x2)
  - ➢ 1,5 mL Eppendorf tubes (x4)
  - ➢ flask TC 75 cm2 (x1)
  - ➢ 10 uL pipette tips (x9)
  - ➢ 1 mL pipette tips (x2)
  - ➢ filters (x2)
  - ➢ syringe 10ml (x2)
  - ➢ pen/strep (1 mL)
  - ➢ DMEM 1X (86,5 mL)
  - ➢ FBS 1X (12,5 mL)
  - ➢ DMEM FBS 15% 1% p/s (16 mL)
  - ➢ DMEM FBS 10% 1% p/s (2 mL)
  - ➢ Trypsin-EDTA (1 mL)
  - ➢ PBS 1x (1,5 mL)
  - ➢ Trypan Blue (40 uL)
- **Method / Protocol Reference**
  - ➢ In-house protocol for cell passaging
- **Sample / Cell Line**
  - ➢ cell line: A-549

Observations & Results

- **Observations / Notes**

- The color of the medium had changed to a paler pink-orange, indicating that the cells had started to metabolise and that the pH of their environment was changing.
- The surface the cells had taken up was around 70-80%.
- Cells with previously observed unusual morphologies were still present, but at a lower percentage.

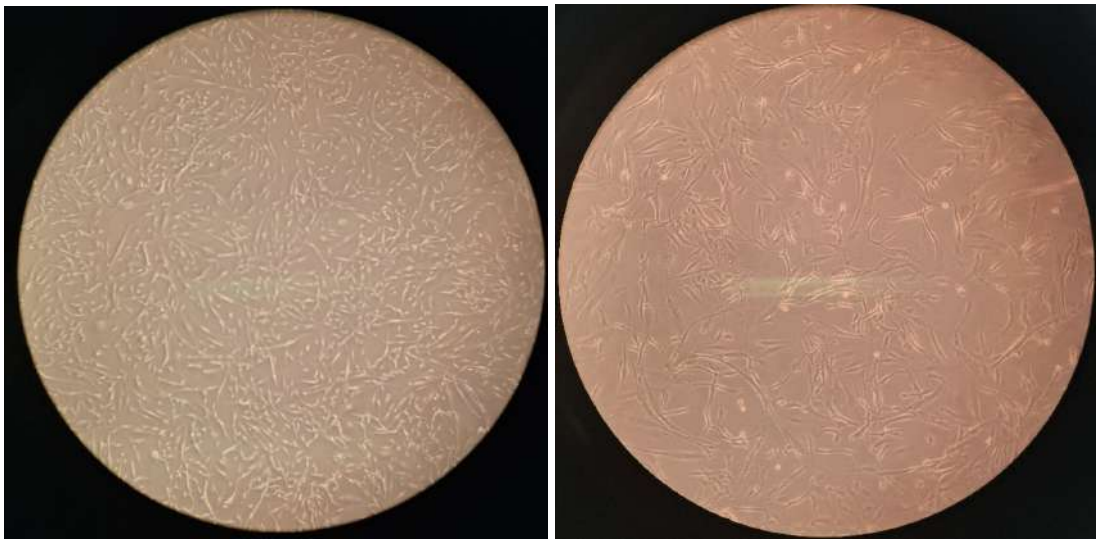


Figure x: Representative pictures of the T-25 flask before the addition of trypsin
Left: 5x focus, Right: 10x focus.

- The residual medium had a volume of 5,9 mL.
- 1mL of trypsin-EDTA was added and after a 5' incubation it was deactivated by adding 2 mL of full medium with 10% FBS → final volume: 3 mL
  - ⅓ of the flask's volume was used for reculture in the T-25 flask (1 mL)
  - ⅔ of the flask's volume was used for passaging to the T-75 flask (2 mL)
    *when removing the flask from the incubator at this step, the cap was screwed shortly after removal and not inside the incubator, so extra careful observation is necessary over the following days.
    **the flask was observed and a small percentage of cells was left behind, so the T-25 flask now contains a bit more than ⅓ of the cells.
- After centrifuging the Falcons containing the volumes above, a pellet was present in both Falcons.
  - The pellet of the Falcon that contained 2 mL was resuspended in 10 mL of complete growth medium supplemented with 15% FBS. Due to a handling error, a quantity of 0,5 mL was lost. The remaining 9,5 mL were transferred to the T-75 cell culture flask.
  - The pellet of the Falcon that contained 1 mL was resuspended in 6 mL of complete growth medium supplemented with 15% FBS, and transferred to the T-25 cell culture flask.

Trypan blue staining:

- During the cell counting step, we performed trypan blue staining on two different aliquots:

- ❖ 1 aliquot of the old medium from the flask
- ❖ 1 aliquot of the flask's volume after the deactivation of trypsin
  *both aliquots were taken after performing up and down thoroughly

- Cells were present in the Neubauer chamber from the aliquot containing the old medium from the flask. One live cell and one dead cell were observed within the designated Neubauer squares.

Culture Medium

A549_T-25_MP	live	dead	Avg live	Avg dead	all	% Viability	cell/ml
5	1	1	0,25	0,25	0,5	50	**5000**
6	0	0					
7	0	0					
8	0	0					
						TOTAL C.N	29500

- Cells were present in the Neubauer chamber from the aliquot containing volume from the flask after the deactivation of trypsin.

A549_T-25_MP	live	dead	Avg live	Avg dead	all	% Viability	cell/ml
5	13	1	7	1	8	87,5	**140000**
6	6	1					
7	4	0					
8	5	2					
						TOTAL C.N	420000

- **Result Summary**
  - The cells were observed, and the abnormal findings were documented with representative photos.
  - After the observation, we decided that the cells were ready for passaging.
  - Trypan Blue staining on the spent growth medium revealed that some of the suspended cells were live.
  - Both flasks were placed back in the incubator at 37C, 5% CO2, and will be monitored closely over the next days.

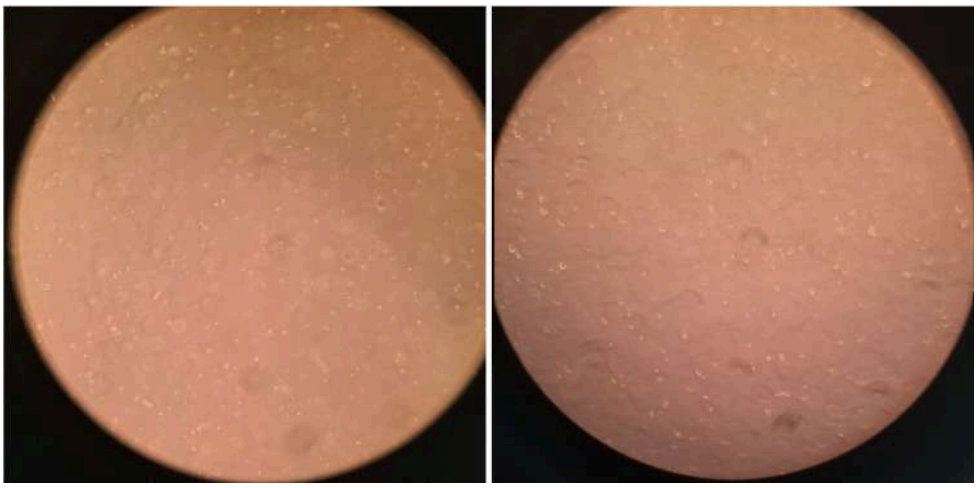


Figure x: Representative pictures of the cell culture flasks after passaging in 5x focus
Left: T-25 cell culture flask, Right: T-75 cell culture flask.

JULY 24TH 2026

Overview

- **Date** – Friday, July 24th 2026
- **Time (Start–End)** – 9:05 - 10:30
- **Name / Initials** – Erriketti Moumka (EM)
- **Shadowing** - Margarita Papastergiou, Alexandros Vlachos, Melita Lampropoulou
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Observation of A-549 cells
- **Objective** – The purpose of this day's experiment was to observe the A-549 cells in the T-25 and T-75 cell culture flasks, and to assess the complete media we prepared yesterday.

Experimental documentation

- **Materials / Reagents Used**
  - ➢ Trypan Blue (30 uL)
  - ➢ 1 mL pipette tips (x3)
  - ➢ 10 uL pipette tips (x6)

Observations & Results

- **Observations / Notes**
  - The color of the medium in both flasks was changed to a paler pink, indicating that the cells had begun to metabolise.
  - The surface the cells had taken up was around 40% in the T-25 flask, and around 30% in the T-75 flask.
  - Following yesterday's passaging procedure, most of the cells had settled and formed a monolayer.
  - The unusual cell morphologies observed the previous days were still present in similar low abundance. Upon retroactive observation, the same morphological findings can be observed in images taken of A-549 cells in 10x focus from 16/07 onwards. Moving forward we will revisit images from our cell cultures in previous months to assess whether these findings were also present then.

- **Result Summary**
  - The cells in both flasks were observed.
  - After the observation, we decided not to change the medium in either flask.
  - The complete media we prepared yesterday were observed under the microscope with Trypan Blue staining, and no abnormal findings were observed.

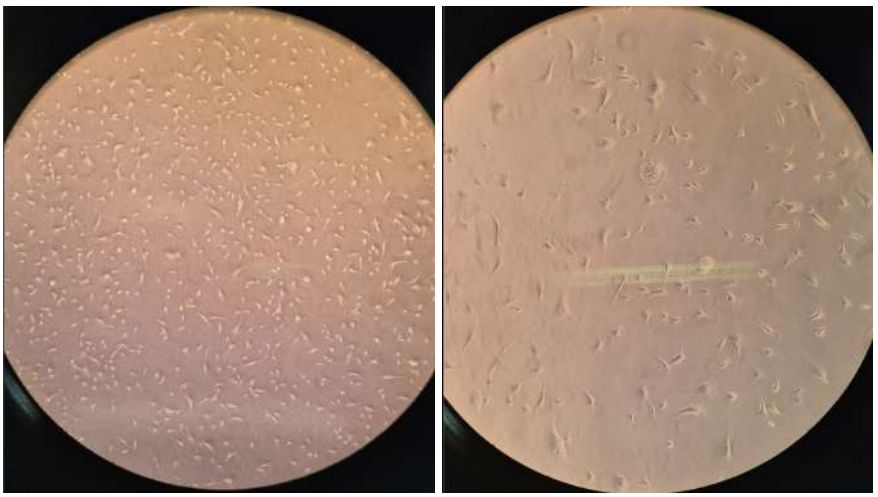


Figure x: Representative image of the T-25 flask under microscope, A-549 cells in 5x focus (left) and 10x focus (right)

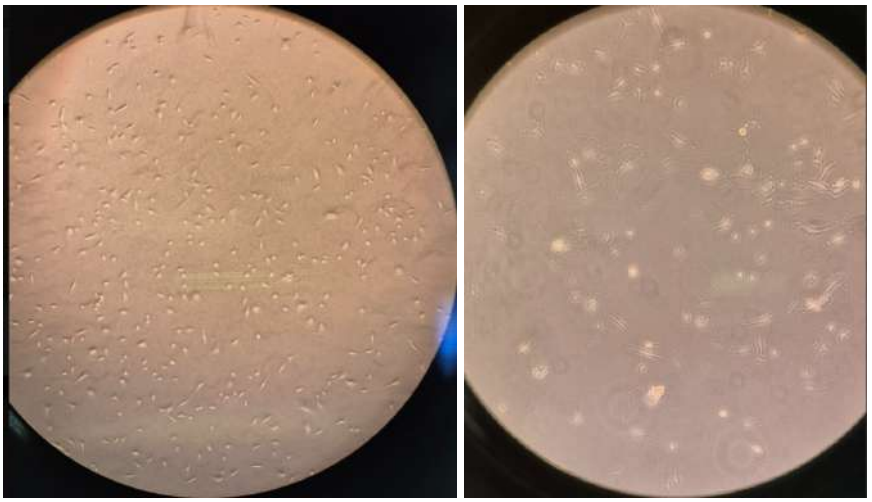


Figure x: Representative image of the T-75 flask under microscope, A-549 cells in 5x focus (left) and 10x focus (right)

JULY 25TH 2026

Overview

- **Date** – Saturday, July 25th 2026
- **Time (Start–End)** – 9:10 - 10:50
- **Name / Initials** – Erriketti Moumka (EM)
- **Shadowing** - Margarita Papastergiou, Alexandros Vlachos, Taxiarchis Kosmas
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Observation of A-549 cells and medium change
- **Objective** – The purpose of this day's experiment was to observe the A-549 cells in the T-25 and T-75 cell culture flasks, and to change the medium. We also performed Trypan Blue staining on the spent medium.

Experimental documentation

- **Materials / Reagents Used**
  - ➢ Trypan Blue (20 uL)
  - ➢ 1 mL pipette tips (x2)
  - ➢ 10 uL pipette tips (x6)
  - ➢ 1,5 mL Eppendorf tubes (×2)
  - ➢ 15 mL Falcon tube (x1)
  - ➢ 30 mL Falcon tube (x1)
  - ➢ 5 mL stripettes (x2)
  - ➢ 10 mL stripettes (x3)
  - ➢ DMEM FBS 15% 1% P/S (14,5 mL)

Observations & Results

- **Observations / Notes**
  - The color of the medium in both flasks was changed to a paler pink-orange, indicating that the cells had begun to metabolise.
  - The surface area the cells had taken up was around 50% in the T-25 flask, and around 40% (with non-homogeneous distribution) in the T-75 flask.
  - The unusual cell morphologies observed the previous days were still present in similar abundance.
  - Residual medium volume was 5,8 mL in the T-25 flask, and 8,9 mL (out of the initial 9,5 mL) in the T-75 flask.

- **Result Summary**
  - The cells in both flasks were observed, and the medium was changed.
  - The medium was changed in both flasks and replaced with DMEM FBS 15% 1% P/S. The final volume was 6 mL (5,5 mL new and 0,5 mL old media) in the T-25, while it was 10 mL (9 mL old and 1 mL new media) in the T-75 flask.
  - Before discarding the old medium in either flask, 1 mL aliquots were taken for Trypan Blue staining. With the Neubauer chamber, cells were observed in both aliquots.

T25 MEDIUM

A549_T-25_MP	live	dead	Avg live	Avg dead	all	% Viability	cell/ml
5	2	0	1	0,5	1,5	66,66666667	20000
6	1	0					
7	1	0					
8	0	2					
						TOTAL C.N	120000

T75 MEDIUM

A549_T-25_MP	live	dead	Avg live	Avg dead	all	% Viability	cell/ml
5	0	0	0,75	0	0,75	100	15000
6	0	0					
7	0	0					
8	3	0					
						TOTAL C.N	142500

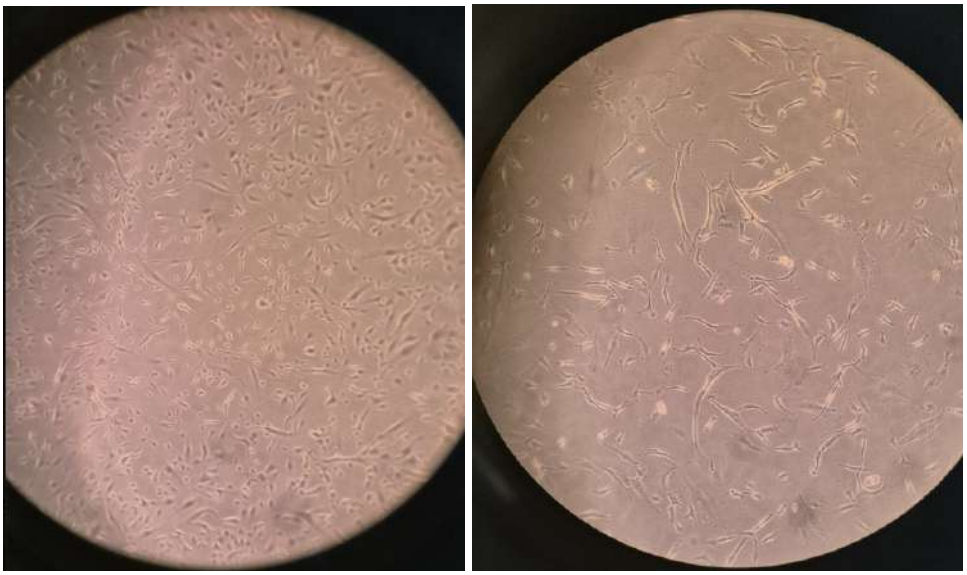


Figure x: Representative image of the T-25 flask under microscope before medium change, A-549 cells in 5x focus (left) and 10x focus (right)

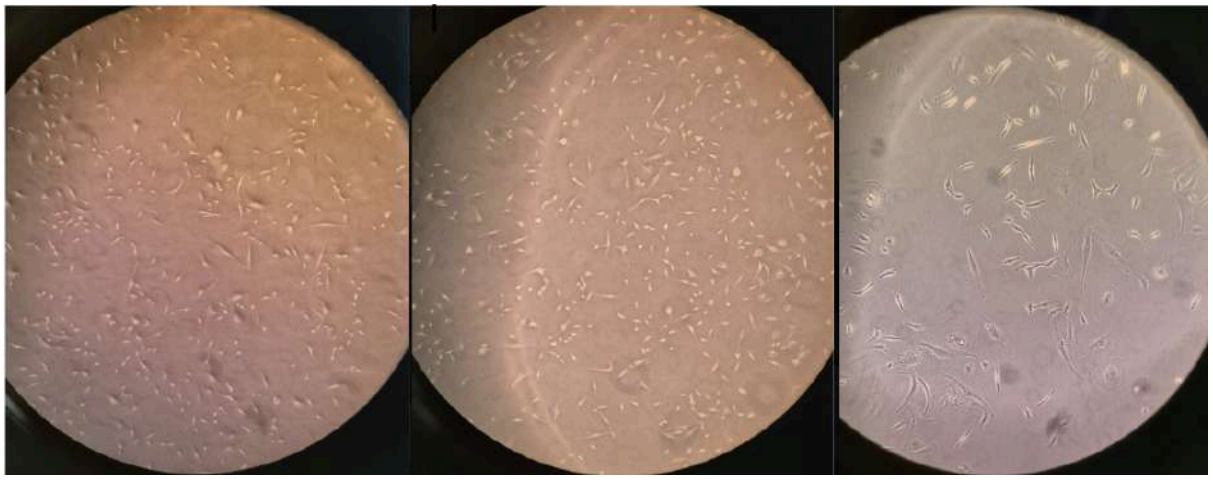


Figure x: Representative image of the T-75 flask under microscope before medium change, A-549 cells in 5x focus (left, middle) and 10x focus (right)

JULY 26TH 2026

Overview

- **Date** – Sunday, July 26th 2026
- **Time (Start–End)** – 9:40 - 10:20
- **Name / Initials** – Erriketti Moumka (EM)
- **Shadowing** - Margarita Papastergiou, Alexandros Vlachos, Ilias Theocharous
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Observation of A-549 cells
- **Objective** – The purpose of this day's experiment was to observe the A-549 cells in the T-25 and T-75 cell culture flasks, and to assess whether we could begin the cell seeding for the biological replicate of the nutlin-3a experiment.

Experimental documentation

- **Materials / Reagents Used**

  -

Observations & Results

- **Observations / Notes**
  - The color of the medium in both flasks was changed to a paler pink, indicating that the cells had begun to metabolise.
  - The surface area the cells had occupied was around 65% in the T-25 flask and around 45% in the T-75 flask.
  - The unusual cell morphologies observed the previous days were still present in similar low abundance.
- **Result Summary**
  - The cells in both flasks were observed.
  - After the observation, we decided to perform the cell seeding tomorrow, after observing both flasks anew.

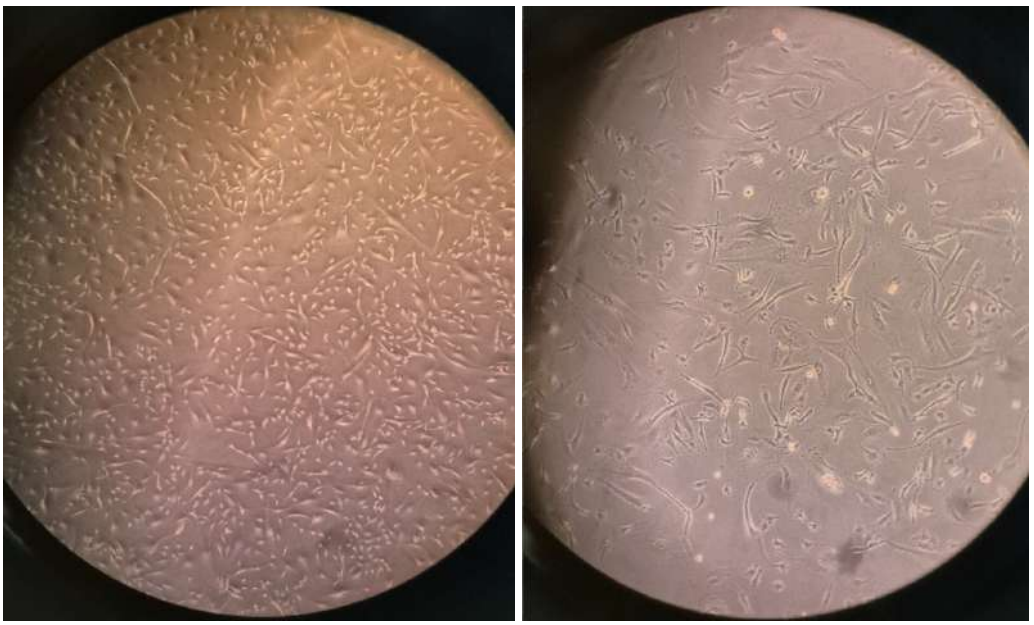


Figure x: Representative image of the T-25 flask under microscope, A-549 cells in 5x focus (left) and 10x focus (right)



Figure x: Representative image of the T-75 flask under microscope, A-549 cells in 5x focus (left) and 10x focus (right)

JULY 27TH 2026

Overview

- **Date** – Monday, July 27th 2026
- **Time (Start–End)** – 9:00 - 13:10
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Melita Lampropoulou, Errika Moumka, Alexandros Vlachos
- **Project / Subproject** – Cellular effect of Nutlin-3a in A-549 cell line (day 0/5)
- **Experiment / Task Title** – Seeding of cells for Nutlin-3a treatment and reculture of A-549 cells.
- **Objective** – The purpose of today's experiment was to seed A-549 cells in a 96-well plate for the Nutlin-3a treatment that was planned for the following days.

Experimental documentation

- **Materials / Reagents Used**
  - ➢ stripettes 10 mL (x3)
  - ➢ 96-well plate (x1)
  - ➢ stripettes 5 mL (x16)
  - ➢ DMEM FBS 10% 1% p/s (8 mL)
  - ➢ DMEM FBS 15% 1% p/s (15,9 mL)
  - ➢ Trypsin - EDTA (0.005%) (4 mL)
  - ➢ PBS 1x (3 mL)
  - ➢ 30 mL Falcon (x2)
  - ➢ 15 mL Falcon (x2)
  - ➢ 1,5 mL tubes (x3)
  - ➢ 1000 µL tips (x8)
  - ➢ 200 µL tips (x33)
  - ➢ 20 µL tips (x8)
  - ➢ Trypan Blue (50 µL)
- **Method / Protocol Reference**
  - ➢ In-house protocol for changing the medium
  - ➢ Cellular effect of Nutlin-3a in the A-549 cell line protocol
- **Sample / Cell Line**
  - ➢ Cell line: A-549

Observations & Results

- **Pre-seeding**
  - The color of the medium had changed from magenta to a paler pink-orange, indicating that the cells metabolised and that the pH of their environment had changed.
  - The surface the cells had taken up was around 80% in the T-25 Flask and around 60% in the T-75 Flask.
  - Each well would have 12.500 cells/well => each timepoint would have 6 different conditions in 2 TRs => 12 wells/timepoint * 4 timepoints = 48 wells

=> 48 wells * 12.500 cells = **600.000 cells needed in total + 70.000 buffer zone => 670.000 cells**

During seeding

- After the centrifugation step, pellets were present in both tubes.
- The pellet was resuspended in full medium:
  - T-25 flask: 0,5 mL
  - T-75 flask: 1 mL
- During the seeding process, some bubbles were created in a few wells.

Result Summary

- The cells were observed, and no abnormalities were noted.
- All technical replicates showed similar morphology and cell density.
- We didn't have enough cell suspension to make 2 technical replicates for the 40 µM condition; that's why we settled for 1 TR for this condition.
  - Every condition except the last one had 2 TRs, and all wells had a final volume of 200 µL.

JULY 28TH 2026

Overview

- **Date** – Tuesday, July 27th 2026
- **Time (Start–End)** – 9:10 - 15:10
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Melita Lampropoulou, Erriketti Moumka, Alexandros Vlachos
- **Project / Subproject** – Cellular effect of Nutlin-3a in A-549 cell line (day 1/5)
- **Experiment / Task Title** – Treatment of cells with Nutlin-3a, observation of flask of A-549 cells, medium preparation.
- **Objective** –The purpose of today's experiment was to treat A-549 cells with Nutlin-3a at different concentrations.

Experimental documentation

- **Materials / Reagents Used**
  - ➢ stripettes 5 mL (x1)
  - ➢ DMEM FBS 15% 1% p/s (23,2 mL)
  - ➢ 30 mL Falcon (x1)
  - ➢ 15 mL Falcon (x5)
  - ➢ 1,5 mL tubes (x4)
  - ➢ 2 mL tubes (x1)
  - ➢ 1000 µL tips (x15)
  - ➢ 200 µL tips (x51)
  - ➢ 20 µL tips (x4)
- **Method / Protocol Reference**
  - ➢ Cellular effect of Nutlin-3a in the A-549 cell line protocol
- **Sample / Cell Line**
  - ➢ Cell line: A-549

Observations & Results

- **Pre-treatment**
  - The color of the medium had changed from magenta to a paler pink-orange, indicating that the cells metabolised and that the pH of their environment had changed.
  - The cells in most wells had covered the entire surface.
  - Some wells had clusters of cells.

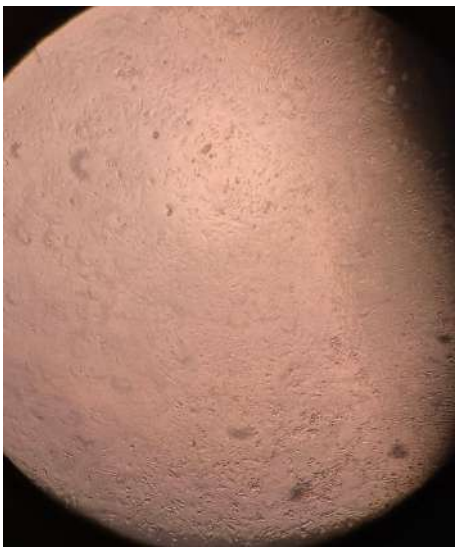


Figure x: Representative picture of a well from the 96-well plate, where clusters are present in the center of the well.



Figure x: Representative picture of the 96-well plate, 24 hours post-seeding.

- **During-treatment**
  - The master mixes were created based on the following matrix:

Condition	Nutlin-3a (2mM stock) (µL)	Nutlin-3a (10 mM stock) (µL)	Full Medium (µL)	DMSO (µL)
c	-	0	2390,4	9,6
2,5 µM	3	-	2397	-
5 µM	6	-	2394	-
10 µM	-	2,4	2397,6	-
20 µM	-	4,8	2.395,2	-
40 µM	-	6,4	1593,6	-

    - The master mixes for conditions c, 2.5, 5, 10, and 20 μM were prepared in a 15 mL Falcon, and the 40 μM condition in a 2 mL tube.
    - 200 μL of the master mix was shared in each well, based on the condition.
    - Each master mix had 800 μL as a buffer zone, in case any pipetting errors occurred.
- **Post-treatment**
    - After the treatment, some wells showed detached cells, and we had trouble focusing on them with the microscope.

Result Summary

- The cells were observed, and no abnormalities were noted.
- All technical replicates showed similar morphology and cell density.

JULY 29TH 2026

Overview

- **Date** – Wednesday, July 29th 2026
- **Time (Start–End)** – 9:10 - 15:00
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Melita Lampropoulou, Erriketti Moumka, Alexandros Vlachos
- **Project / Subproject** – Cellular effect of Nutlin-3a in A-549 cell line (day 2/5)
- **Experiment / Task Title** – Cell viability assay of A-549 cells with Nutlin-3a, 24 h post-treatment and reculture.
- **Objective** – The purpose of today's experiment was to check the cell viability of A-549 cells treated with Nutlin-3a at different concentrations, 24 hours post-treatment.

Experimental documentation

- **Materials / Reagents Used**
  - ❖ 15 mL Falcon (x1)
  - ❖ 30 mL Falcon (x1)
  - ❖ 10 uL tips (x46)
  - ❖ 200 uL tips (x48)
  - ❖ 1 mL tips (x6)
  - ❖ 1,5 mL tubes (x54)
  - ❖ Trypan blue (180 uL)
  - ❖ PBS 1x (4,2 mL)
  - ❖ Trypsin-EDTA (2,4 mL)
  - ❖ DMEM, 10% FBS, 1% p/s (4,8 mL)
- **Method / Protocol Reference**
  - ➢ Cellular effect of Nutlin-3a in the A-549 cell line protocol
- **Sample / Cell Line**
  - ➢ Cell line: A-549

Observations & Results

- The color of the medium had changed from magenta to a paler pink-orange, indicating that the cells had started to metabolise and that the pH of their environment was changing, in the 96-well plate.
- The pellets were stored at -80oC at the pharmacology building inside the blue box.
- Initially measured the volumes of the wells and distributed them into the corresponding tubes, including preparation of the 3rd TR/condition.
- Detached the cells after washing with PBS 1x (50 μL/well), using trypsin (50 μL/well).
- Observed that in some wells not all cells had detached to the same extent; however, tapping the plate confirmed cell movement (0 μM TR1, 5 μM TR2, 10 μM TR1, 40 μM TR).

- Performed an additional trypsin incubation on these specific wells, since the volumes from the remaining wells had already been removed.
- Note: at this step, PBS 1x should have been added instead of trypsin, to avoid further stressing the cells.
- Collected 40 µL from each tube for Trypan Blue (TB) measurements (performed by me).
- Pooled the remaining volume of each tube per condition into a new tube for pellet preparation (performed by Erriketti and Alexandros; 6 pellets in total).
- Performed 3 Trypan Blue measurements per condition; all data have been recorded in our Excel file.



96-well plate 24 hours post treatment

JULY 30TH 2026

Overview

- **Date** – Thursday, July 30th 2026
- **Time (Start–End)** – 10:40 - 16:40
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Melita Lampropoulou, Taxiarchis Kosmas, Ilias Theocharous, Alexandros Vlachos
- **Project / Subproject** – Cellular effect of Nutlin-3a in A-549 cell line (day 3/5)
- **Experiment / Task Title** – Cell viability assay of A-549 cells with Nutlin-3a, 48h post-treatment and reculture.
- **Objective** – The purpose of today's experiment was to check the cell viability of A-549 cells treated with Nutlin-3a at different concentrations, 48 hours post-treatment.

Experimental documentation

- **Materials / Reagents Used**
  - ❖ 15 mL Falcon (x1)
  - ❖ 10 uL tips (x52)
  - ❖ 200 uL tips (x62)
  - ❖ 1 mL tips (x6)
  - ❖ 1,5 mL tubes (x54)
  - ❖ Trypan blue (180 uL)
  - ❖ PBS 1x (4,2 mL)
  - ❖ Trypsin-EDTA (2,4 mL)
  - ❖ DMEM, 10% FBS, 1% p/s (4,8 mL)
- **Method / Protocol Reference**
  - ➢ Cellular effect of Nutlin-3a in the A-549 cell line protocol
- **Sample / Cell Line**
  - ➢ Cell line: A-549

Observations & Results

- In the 96-well plate, cells were observed in all wells, with aggregates present in some of the last wells.
- Supernatant was collected and distributed to the corresponding TR and the 3rd TR, with most volumes remaining close to 200 µL.
- Washing with PBS 1× followed, without cell detachment, then trypsinization (50 µL/well, up to 4 minutes incubation).
- After trypsin inactivation with medium, cells were collected into new tubes.
- Where cells remained attached, an additional pressurized PBS wash was applied, ensuring full recovery of the cells. This step was very effective and eliminated the need for a second trypsin treatment.
- The cell suspension was recombined with the corresponding supernatant, and 40 µL were taken from each tube (supernatant + monolayer cells) after thorough up-and-down mixing.

- The remaining volume of each TR was pooled per condition for pellet preparation.
- Trypan Blue measurements were performed with shadowing support, while pellet preparation was carried out by other team members, with additional shadowing and assistance with sterilization procedures.
- Pellets were stored at -80°C in the molecular lab.

JULY 31TH 2026

Overview

- **Date** – Friday, July 31th 2026
- **Time (Start–End)** – 11:00 - 15:20
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Ilias Theocharous, Erriketti Moumka, Alexandros Vlachos
- **Project / Subproject** – Cellular effect of Nutlin-3a in A-549 cell line (day 4/5)
- **Experiment / Task Title** – Cell viability assay of A-549 cells with Nutlin-3a, 72h post-treatment and reculture.
- **Objective** – The purpose of today's experiment was to check the cell viability of A-549 cells treated with Nutlin-3a at different concentrations, 72 hours post-treatment.

Experimental documentation

- **Materials / Reagents Used**
  - ❖ 15 mL Falcon (x1)
  - ❖ 30 mL Falcon (x1)
  - ❖ 10 uL tips (x48)
  - ❖ 200 uL tips (x12)
  - ❖ 1 mL tips (x6)
  - ❖ 1,5 mL tubes (x54)
  - ❖ 2 mL tubes (x2)
  - ❖ Trypan blue (200 uL)
  - ❖ PBS 1x (4,2 mL)
  - ❖ Trypsin-EDTA (2,4 mL)
  - ❖ DMEM, 10% FBS, 1% p/s (4,8 mL)
- **Method / Protocol Reference**
  - ➢ Cellular effect of Nutlin-3a in the A-549 cell line protocol
- **Sample / Cell Line**
  - ➢ Cell line: A-549

Observations & Results

- Today marked the completion of 72 hours post-treatment with N-3a, which included cell observation, sample collection, Trypan Blue measurements, and pellet preparation, carried out by the team.
- In the 96-well plate, cells were confirmed present in all wells, with aggregates also observed in the last wells.
- Supernatant was collected and distributed to the corresponding TR and the 3rd TR, with most volumes maintained close to 200 µL.
- Washing with PBS 1× followed, with no cell detachment observed, then trypsinization (50 µL/well, up to 4 minutes incubation).
- After trypsin inactivation with complete medium, cells were collected into new tubes.
- In wells where cells remained attached, a pressurized PBS wash was applied, allowing full recovery of the cells without requiring a second trypsin application.

- After thorough up-and-down pipetting, the cell suspension was recombined with the corresponding supernatant.
- 40 µL (supernatant + monolayer cells) were retained from each tube, while the remaining volume of each TR was pooled per condition for pellet preparation.
- Trypan Blue measurements were performed with shadowing support, and pellet preparation was carried out by other team members, with additional shadowing involved.
- Pellets were stored at -80°C in the molecular lab.



96-well plate 72 hours post treatment

AUGUST 1ST 2026

Overview

- **Date** – Saturday, August 1st 2026
- **Time (Start–End)** – 10:50 - 15:45
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Melita Lampropoulou, Taxiarchis Kosmas, Ilias Theocharous, Alexandros Vlachos, Erriketti Moumka
- **Project / Subproject** – Cellular effect of Nutlin-3a in A-549 cell line (day 5/5)
- **Experiment / Task Title** – Cell viability assay of A-549 cells with Nutlin-3a, 96h post-treatment and reculture.
- **Objective** – The purpose of today's experiment was to check the cell viability of A-549 cells treated with Nutlin-3a at different concentrations, 96 hours post-treatment.

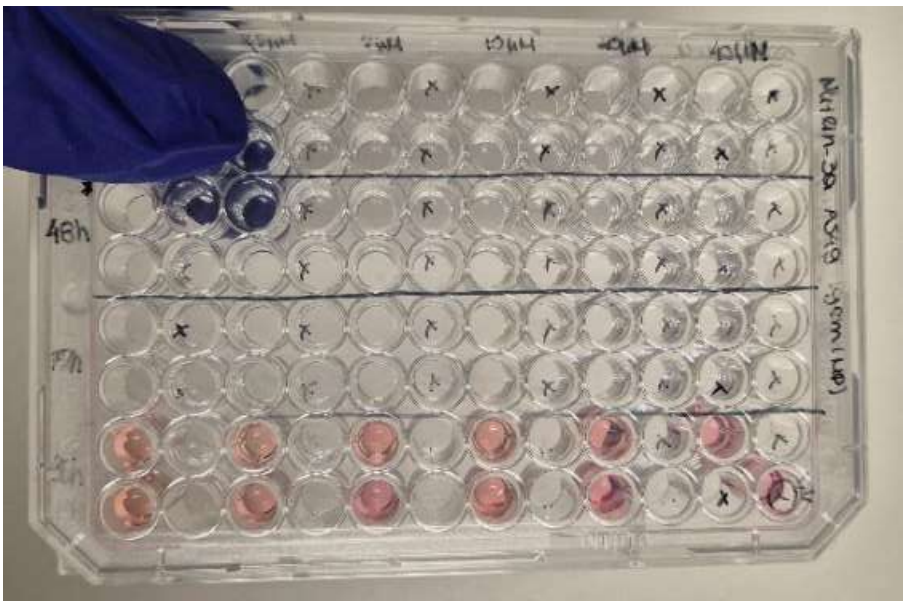
Experimental documentation

- **Materials / Reagents Used**
  - ❖ 15 mL Falcon (x1)
  - ❖ 30 mL Falcon (x1)
  - ❖ 10 uL tips (x50)
  - ❖ 200 uL tips (x42)
  - ❖ 1 mL tips (x6)
  - ❖ 1,5 mL tubes (x54)
  - ❖ Trypan blue (210 uL)
  - ❖ PBS 1x (4,2 mL)
  - ❖ Trypsin-EDTA (2,4 mL)
  - ❖ DMEM, 10% FBS, 1% p/s (4,8 mL)
- **Method / Protocol Reference**
  - ➢ Cellular effect of Nutlin-3a in the A-549 cell line protocol
- **Sample / Cell Line**
  - ➢ Cell line: A-549

Observations & Results

- Today marked the completion of the final timepoint of the experiment (96 hours post-treatment with N-3a). Procedures performed included cell observation, sample collection, cell counting with Trypan Blue, and pellet preparation, carried out by the team.
- Observation of the 96-well plate confirmed the presence of cells in all wells, with cellular aggregates also observed in some of the last wells.
- Supernatant was collected from each well and distributed into the corresponding tubes (TR and 3rd TR).
- Washing with PBS 1× followed, with no cell detachment observed, and then trypsinization was performed with 50 µL of trypsin per well for up to 4 minutes.
- After trypsin inactivation with complete medium, cells were transferred into new tubes.

- In wells where cells remained attached, a pressurized PBS wash was applied, achieving full recovery of the cells without requiring a second trypsinization.
- After sufficient up-and-down pipetting, the cell suspension was combined with the corresponding supernatant.
- 40 µL (supernatant and monolayer cells) were retained from each tube, while the remaining volume of each technical replicate was pooled per condition for pellet preparation.
- Cell counting with Trypan Blue was performed with shadowing support during the procedure.
- Pellet preparation was carried out by other team members, with additional shadowing participation.
- All pellets were stored at -80°C in the molecular laboratory



96-well plate 96 hours post treatment

AUGUST 2ND 2026

Overview

- **Date** – Sunday, August 2nd 2026
- **Time (Start–End)** – 10:45 - 13:25
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Melita Lampropoulou, Alexandros Vlachos, Erriketti Moumka
- **Project / Subproject** – Cellular effect of Nutlin-3a in A-549 cell line (day 1/5).
- **Experiment / Task Title** – Seeding of cells for Nutlin-3a treatment.
- **Objective** – The purpose of today's experiment was to seed A-549 cells in a 96-well plate for the Nutlin-3a treatment that was planned for the following days

Experimental documentation

- **Materials / Reagents Used**
  - ❖ 96-well plate (x1)
  - ❖ 15 mL Falcon (x2)
  - ❖ 50 mL Falcon (x1)
  - ❖ 10 uL tips (x11)
  - ❖ 200 uL tips (x21)
  - ❖ 1 mL tips (x8)
  - ❖ 1,5 mL tubes (x3)
  - ❖ 5 mL stripettes (x9)
  - ❖ 10 mL stripettes (x5)
  - ❖ Trypan blue (50 uL)
  - ❖ PBS 1x (6 mL)
  - ❖ Trypsin-EDTA (3,5 mL)
  - ❖ DMEM, 10% FBS, 1% p/s (23,8 mL)
- **Method / Protocol Reference**
  - ➢ Cellular effect of Nutlin-3a in the A-549 cell line protocol
  - ➢ In-house medium change
- **Sample / Cell Line**
  - ➢ Cell line: A-549

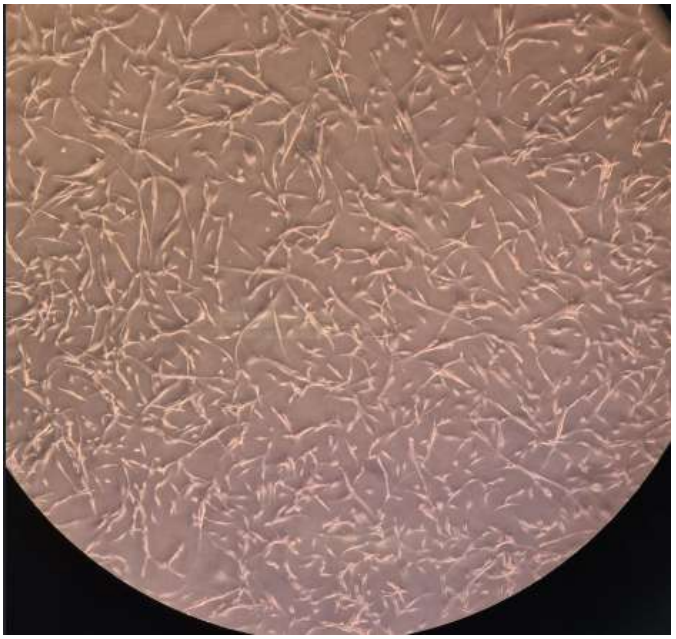
Observations & Results

- Seeding was performed using the A-549 cell line.
- Cells were seeded at a density of 12,500 cells/well in a 96-well plate.
- The source T25 flask was at approximately 90% confluency at the time of seeding.
- Some cell aggregates were observed in the flask prior to seeding.

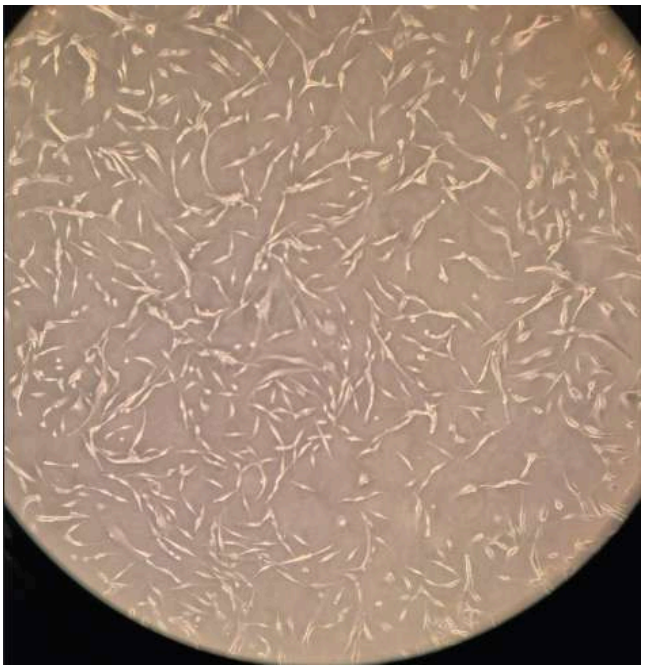
Observations & Results

- **Pre-seeding**
  - The color of the medium had changed from magenta to a paler pink-orange, indicating that the cells metabolised and that the pH of their environment had changed.

- The surface the cells had taken up was around 85% in the T-25 Flask and around 60% in the T-75 Flask.



T-25 Flask (5x focus)



T-75 Flask (5x focus)

- Each well would have 12.500 cells/well => each timepoint would have 6 different conditions in 2 TRs => 12 wells/timepoint * 4 timepoints = 48 wells => 48 wells * 12.500 cells = **600.000 cells needed in total + 80.000 buffer zone => 680.000 cells**

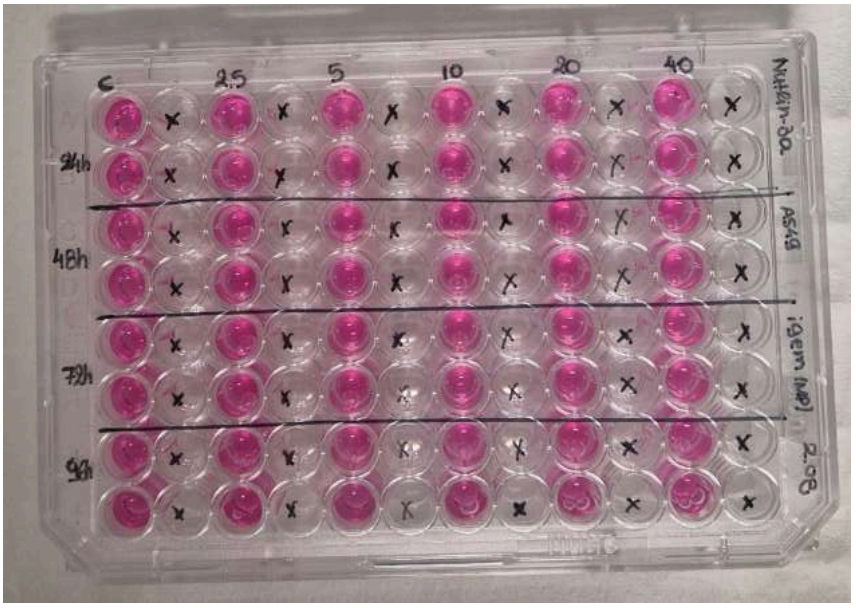
During seeding

- After the centrifugation step, pellets were present in both tubes.
- The pellet was resuspended in full medium:
  - T-25 flask: 0,5 mL

- ❖ T-75 flask: 1 mL
- During the seeding process, some bubbles were created in a few wells.
- Cell seeding was performed by dispensing half the volume in alternating directions, column by column.

Result Summary

- The cells were observed, and no abnormalities were noted.
- All technical replicates showed similar morphology and cell density.



96-well plate, immediately after the seeding step was completed.

AUGUST 3RD 2026

Overview

- **Date** – Monday, August Erd2026
- **Time (Start–End)** – 10:25 - 13:05
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Ilias Theocharous, Alexandros Vlachos, Erriketti Moumka
- **Project / Subproject** – Cellular effect of Nutlin-3a in A-549 cell line (day 1/5).
- **Experiment / Task Title** – Treatment of A-549 cells with Nutlin-3a.
- **Objective** – The purpose of today's experiment was to treat A-549 cells with Nutlin-3a at different concentrations.

Experimental documentation

- **Materials / Reagents Used**
  - ❖ 15 mL Falcon (x7)
  - ❖ 10 uL tips (x8)
  - ❖ 200 uL tips (x38)
  - ❖ 1 mL tips (x22)
  - ❖ DMEM, 10% FBS, 1% p/s (13,6 mL)
  - ❖ DMSO (9,6 uL)
  - ❖ Nutlin-3a 10 mM (stock) (13,6 µL)
  - ❖ Nutlin-3a 2 mM (stock) (9 µL)
- **Method / Protocol Reference**
  - ➢ Cellular effect of Nutlin-3a in the A-549 cell line protocol
- **Sample / Cell Line**
  - ➢ Cell line: A-549

Observations & Results

- **Pre-treatment**
  - The wells were observed, and more than 60% of the surface was covered, allowing us to proceed with the treatment.
    - Observations: Slightly higher cell density was observed in the 2.5 µM condition across all timepoints, and less so in the 40 µM condition across all timepoints.
    - In C TR1 & TR2 wells, we noticed a spot void of cells in the lower hemisphere of the well.
    - Most of the bubbles observed yesterday were no longer present.
  - Each master mix was comprised of: Nutlin-3a and full medium (except the 0 µM, which instead of N-3a it contained DMSO).
  - The master mixes were prepared based on the following matrix:

Condition	Nutlin-3a, 2 mM stock (µL)	Nutlin-3a, 10 mM stock (µL)	Full Medium (µL)	DMSO (µL)
0 µM	–	0	2390.4	9.6
2.5 µM	3	–	2397	–
5 µM	6	–	2394	–
10 µM	–	2.4	2397.6	–
20 µM	–	4.8	2395.2	–
40 µM	–	9.6	2390.4	–

- **During the treatment**
  - The final volume of each well was 200 µL. The treatment was shared per condition across all timepoints, in two intervals: 100 µL from the 0 µM TR1 24h condition to the 0 µM TR2 96h, and 100 µL from the 0 µM TR2 96h condition to the 0 µM TR1 24h condition.
- **Post-treatment**
  - The cells were observed, and no abnormalities were noted.

AUGUST 4TH 2026

Overview

- **Date** – Tuesday, August 4th 2026
- **Time (Start–End)** – 10:55 - 21:00
- **Name / Initials** – Erriketti Moumka (EM)
- **Shadowing** - Alexandros Vlachos, Ilias Theocharous
- **Project / Subproject** – Cellular effect of Nutlin-3a in A-549 cell line 3rd Biological Replicate
- **Experiment / Task Title** – Cell viability assay of A-549 cells with Nutlin-3a, 24 h post-treatment and reculture.
- **Objective** – The purpose of today's experiment was to check the cell viability of A-549 cells treated with Nutlin-3a at different concentrations, 24 hours post-treatment, and to change the medium in the T-25 (AV) and T-75 (IT) cell culture flasks.

Experimental documentation

- **Materials / Reagents Used**
  - ➢ 96-well plate (x1)
  - ➢ 15 mL Falcon tubes (x2)
  - ➢ 10 uL tips (x78)
  - ➢ 200 uL tips (x112)
  - ➢ 1 mL tips (x10)
  - ➢ 1.5 mL Eppendorf tubes (x66)
  - ➢ 5 mL stripettes (x2)
  - ➢ 10 mL stripettes (x2)
  - ➢ Trypan Blue (300 uL)
  - ➢ PBS 1X (3.8 mL)
  - ➢ trypsin-EDTA (0.6 mL)
  - ➢ DMEM FBS 10% 1% p/s (15.7 mL)
- **Method / Protocol Reference**
  - ➢ In-house protocol for changing the medium
  - ➢ Cellular effect of Nutlin-3a in the A-549 cell line protocol
- **Sample / Cell Line**
  - ➢ Cell line: A-549

Observations & Results

T-25 cell culture flask

- The color of the medium had changed from magenta to a paler pink-orange, indicating that the cells metabolised and that the pH of their environment had changed.
- Cell confluency was about 60-70%.
- Cells with the previously recorded unusual morphologies were present, but were largely non-adherent.

- The medium was changed with DMEM 10% FBS, to a final volume of 6 ml.

T-75 cell culture flask

- The color of the medium had changed from magenta to a paler pink-orange, indicating that the cells metabolised and that the pH of their environment had changed.
- Cell confluency was about 50-60%, without homogeneous distribution
- No abnormalities were noted
- The medium was changed with DMEM 10% FBS, to a final volume of 10 ml.

N-3a treatment 24 h

- All wells were observed under the microscope and representative photos were taken.
    - Some vacant spots were observed in all wells in the lower hemisphere, but especially in the wells for the following conditions: 0 μM TR1, 0 μM TR2, 20 μM TR2, 40 μM TR1, and 40 μM TR2.
    - In general, the center of the wells showed lower cell density than the sides (with 20 μM TR1 & TR2 being an exception)
    - In the 40 μM condition, the cells appeared to have largely lost their usual morphology, appearing more round and aggregating in small groups.
- Spent medium was collected from each condition for both TRs for the 24h timepoint and the volume was assessed by reverse pipetting.
- The PBS wash was collected from each well and pooled with the rest of the cellular contents of each corresponding condition because it seemed as if a non-negligible amount of cells had detached in the time they remained in PBS.
- After trypsinization and harvesting, cells remained in 0 μM TR1 and 40 μM TR1, so a second, forceful PBS wash (100 μM) was performed in order to detach and harvest the remaining cells.
- All cellular contents from a certain condition and TR were pooled together after half was set aside from both TR1 and TR2 in order to form TR3.
    - A volume of 40 μM was collected from each of the three TRs for cell counting. Due to low cell yield, centrifugation was performed and the pellets were resuspended in half the volume. All cell counts were performed by Ilias Theocharous.
    - The remaining volumes from each condition were pooled and made into cell pellets. The pellets were stored at -80oC at the pharmacology building inside the white box.

AUGUST 5TH 2026

Overview

- **Date** – Wednesday, August 5th 2026
- **Time (Start–End)** – 10:55 - 21:40
- **Name / Initials** – Erriketti Moumka (EM)
- **Shadowing** - Alexandros Vlachos, Ilias Theocharous
- **Project / Subproject** – Cellular effect of Nutlin-3a in A-549 cell line 3rd Biological Replicate
- **Experiment / Task Title** – Cell viability assay of A-549 cells with Nutlin-3a, 48 h post-treatment and reculture.
- **Objective** – The purpose of today's experiment was to check the cell viability of A-549 cells treated with Nutlin-3a at different concentrations, 48 hours post-treatment. Additionally, the cells in the T-25 flask were split for pellet formation and for reculture.

Experimental documentation

- **Materials / Reagents Used**
  - ➢ 15 mL Falcon tubes (x3)
  - ➢ 10 uL tips (x78)
  - ➢ 200 uL tips (x84)
  - ➢ 1 mL tips (x16)
  - ➢ 1.5 mL Eppendorf tubes (x62)
  - ➢ 2 mL Eppendorf tubes (x1)
  - ➢ 5 mL stripettes (x4)
  - ➢ 10 mL stripettes (x1)
  - ➢ Trypan Blue (300 uL)
  - ➢ PBS 1X (5.1 mL)
  - ➢ trypsin-EDTA (1.6 mL)
  - ➢ DMEM FBS 10% 1% p/s (3.2 mL)
- **Method / Protocol Reference**
  - ➢ In-house protocol for changing the medium
  - ➢ Cellular effect of Nutlin-3a in the A-549 cell line protocol
- **Sample / Cell Line**
  - ➢ Cell line: A-549

Observations & Results

T-25 cell culture flask

- The color of the medium had changed from magenta to a paler pink, indicating that the cells metabolised and that the pH of their environment had changed.
- Confluency was about 80%, requiring a split.
- Initial medium volume was 5.9 mL.
- Medium was removed and the cells were washed with 1.5 mL PBS. Cells were then treated with 1 mL of trypsin-EDTA within the safe for cells timeframe and deactivated

with twice the volume of complete medium with 10% FBS. Upon observation, the amount of cells present in the flask following trypsinization and harvesting was negligible.

- ⅓ of the resulting cell suspension was centrifuged and resuspended in 6 mL of fresh complete medium with 10% FBS, to be recultured in the T-25 flask.
- The remaining ⅔ were made into a cell pellet, stored at -80oC at the pharmacology building inside the white box.

T-75 cell culture flask

- The color of the medium had changed from magenta to a paler pink, indicating that the cells metabolised and that the pH of their environment had changed.
- Cell confluency was about 55-65%, without homogeneous distribution
- A cluster of dead cells was observed on the left side of the flask.

N-3a treatment 48 h

- All wells were observed under the microscope and representative photos were taken.
    - Some vacant spots were observed in 20 μM TR1 & 5 μM TR1 wells.
    - In 10 μM TR1, 40 μM TR1 & TR2 distribution of cells appeared to be especially non-homogeneous.
    - In the 20 & 40 μM conditions, the cells appeared to have largely lost their usual morphology, appearing more round and aggregating in small groups.
- Spent medium was collected from each condition for both TRs for the 48h timepoint and the volume was assessed by reverse pipetting.
- The PBS wash was collected only from 40 μM TR1 &TR2 and pooled with the rest of the cellular contents of each corresponding condition because it seemed as if a non-negligible amount of cells had detached in the time they remained in PBS. In some wells the cells appeared to have altered morphology (more round), without observed detachment.
- All cellular contents from a certain condition and TR were pooled together after half was set aside from both TR1 and TR2 in order to form TR3.
    - A volume of 40 μM was collected from each of the three TRs for cell counting. Due to low cell yield, centrifugation was performed and the pellets were resuspended in half the volume. All cell counts were performed by Ilias Theocharous.
    - The remaining volumes from each condition were pooled and made into cell pellets. The pellets were stored at -80oC at the pharmacology building inside the white box.

AUGUST 6TH 2026

Overview

- **Date** – Thursday, August 6th 2026
- **Time (Start–End)** – 10:15 - 18:35
- **Name / Initials** – Erriketti Moumka (EM)
- **Shadowing** - Alexandros Vlachos, Ilias Theocharous
- **Project / Subproject** – Cellular effect of Nutlin-3a in A-549 cell line 3rd Biological Replicate
- **Experiment / Task Title** – Cell viability assay of A-549 cells with Nutlin-3a, 72 h post-treatment and cell pelleting.
- **Objective** – The purpose of today's experiment was to check the cell viability of A-549 cells treated with Nutlin-3a at different concentrations, 72 hours post-treatment. Additionally, cell pellets were created from the contents of the T-25 and T-75 culture flasks.

Experimental documentation

- **Materials / Reagents Used**
  - ➢ 15 mL Falcon tubes (x3)
  - ➢ 30 mL Falcon tubes (x1)
  - ➢ 10 uL tips (x36)
  - ➢ 200 uL tips (x156)
  - ➢ 1 mL tips (x16)
  - ➢ 1.5 mL Eppendorf tubes (x65)
  - ➢ 2 mL Eppendorf tubes (x2)
  - ➢ 5 mL stripettes (x6)
  - ➢ 10 mL stripettes (x5)
  - ➢ Trypan Blue (300 uL)
  - ➢ PBS 1X (12.1 mL)
  - ➢ trypsin-EDTA (3.6 mL)
  - ➢ DMEM FBS 10% 1% p/s (7.2 mL)
- **Method / Protocol Reference**
  - ➢ In-house protocol for cell pelleting
  - ➢ Cellular effect of Nutlin-3a in the A-549 cell line protocol
- **Sample / Cell Line**
  - ➢ Cell line: A-549

Observations & Results

Cell Pelleting

For the T-25 flask:

- Medium color had changed to a paler pink, indicating that the cells metabolised and that the pH of their environment had changed.
- Confluency was around 20-30%, with non-homogeneous distribution of the cells. Some cells had still not settled following yesterday's reculture.
- The initial medium volume was 6 mL.

- Medium was removed and the cells were washed with 1.5 mL PBS. Cells were then treated with 1 mL of trypsin-EDTA within the safe for cells timeframe and deactivated with twice the volume of complete medium with 10% FBS. Upon observation, the amount of cells present in the flask following trypsinization and harvesting was negligible.

For the T-75 flask:

- Medium color had changed to a pale orange-pink, indicating that the cells metabolised and that the pH of their environment had changed.
- Confluency was around 65%, with non-homogeneous distribution of the cells.
- The initial medium volume was 9.6 mL.
- Medium was removed and the cells were washed with 4 mL PBS. Cells were then treated with 2 mL of trypsin-EDTA within the safe for cells timeframe and deactivated with twice the volume of complete medium with 10% FBS. Upon observation, the amount of cells present in the flask following trypsinization and harvesting was low but a higher density was observed near the cap, so a second, focused wash of the cell suspension was performed to ensure maximum cell detachment.

Both resulting cell suspensions were pooled and homogenised, and the volume was then split in half before centrifugation. After careful aspiration of the supernatants the visible cell pellets were resuspended in 1,5 mL of PBS before the last centrifugation. The pellets were stored at -80oC at the pharmacology building inside the white box.

N-3a treatment 72 h

- All wells were observed under the microscope and representative photos were taken.
    - In 0 μM TR1 & TR2 we observed uncharacteristically rounded cells (attached) & lower confluency than expected when compared with other conditions.
    - In the 20 & 40 μM conditions, the cells appeared to have largely lost their usual morphology, appearing more round and aggregating in small groups.
- Spent medium was collected from each condition for both TRs for the 72h timepoint and the volume was assessed by reverse pipetting.
    - In 0 μM TR1 and TR2 conditions the medium volume was low and there were visible droplets on the lid of the plate, possibly because of rough handling at any point.
    - In 10 μM TR1 condition, the volume exceeded that of 200 μM pointing towards pipetting error during treatment.
- While in PBS, the cells were observed under the microscope. In 0 μM TR1, cell morphology had shifted to more rounded, in contrast with other conditions of TR1. In 40 μM TR1, the number of cells observed was much lower than that during the initial observation, which led us to believe many cells may have detached and been harvested during aspiration of the growth medium.
- The PBS wash was collected only from the second technical replicate of each condition and pooled with the rest of the cellular contents of each corresponding condition, because it seemed as if a non-negligible amount of cells had detached in the time they remained in PBS.
- All cellular contents from a certain condition and TR were pooled together after half was set aside from both TR1 and TR2 in order to form TR3.
    - A volume of 40 μM was collected from each of the three TRs for cell counting. Due to low cell yield, centrifugation was performed and the pellets were

resuspended in half the volume. All cell counts were performed by Ilias Theocharous.

- The remaining volumes from each condition were pooled and made into cell pellets. The pellets were stored at -80oC at the pharmacology building inside the white box.

AUGUST 7TH 2026

Overview

- **Date** – Friday, August 7th 2026
- **Time (Start–End)** – 9:15 - 17:20
- **Name / Initials** – Ilias Theocharous (IT)
- **Shadowing** - Alexandros Vlachos (author of this report)
- **Project / Subproject** – Cellular effect of Nutlin-3a in A-549 cell line 3rd Biological Replicate
- **Experiment / Task Title** – Cell viability assay of A-549 cells with Nutlin-3a, 96 h post-treatment and cell pelleting.
- **Objective** – The purpose of today's experiment was to check the cell viability of A-549 cells treated with Nutlin-3a at different concentrations, 96 hours post-treatment.

Experimental documentation

- **Materials / Reagents Used**
    - 15 mL Falcon tubes (x2)
    - 10 uL tips (x30)
    - 200 uL tips (x126)
    - 1 mL tips (x7)
    - 1.5 mL Eppendorf tubes (x64)
    - Trypan Blue (200 uL)
    - PBS 1X (5.5 mL)
    - trypsin-EDTA (1.2 mL)
    - DMEM FBS 10% 1% p/s (2.4 mL)
- **Method / Protocol Reference**
    - ❖ Cellular effect of Nutlin-3a in the A-549 cell line protocol
- **Sample / Cell Line**
    - ❖ Cell line: A-549

Observations & Results

N-3a treatment 96 h

- All wells were observed under the microscope. Cell confluency appeared to decrease as nutlin-3a concentration increased, except for 5TRs and 10TRs, where confluency remained about the same.
- Spent medium was collected from each condition for both TRs at the 96 h time point, and the volume was assessed by reverse pipetting.
    - In cTR1, cTR2, 2.5TR1, and 2.5TR2, the medium volume was low, and visible droplets were present on the plate lid, possibly due to rough handling at some point.
- The PBS wash was collected from every well (96h) and pooled with the rest of the cellular contents of each corresponding condition, because a significant amount of cells had detached during washing.

- During cell detachment, only in the cTR1 well, the standard trypsin treatment wasn't enough. After the protocol was applied twice, many cells were still attached to the plate. They were eventually scraped and collected.
- All cellular contents from a certain condition and TR were pooled together after half was set aside from both TR1 and TR2 in order to form TR3.
    - A volume of 40 μM was collected from each of the three TRs for cell counting. Due to low cell yield, centrifugation was performed and the pellets were resuspended in half volume. All cell counts were performed by Ilias Theocharous.
    - The remaining volumes from each condition were pooled and made into cell pellets. The pellets were stored at -80oC at the pharmacology building inside the white box.

AUGUST 29TH 2026

Overview

- **Date** – Saturday, August 29th 2026
- **Time (Start–End)** – 9:30 - 11:00
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Alexandros Vlachos, Erriketti Theodora Moumka
- **Project / Subproject** – Preparation for upcoming experiments
- **Experiment / Task Title** – Medium preparation and inventory
- **Objective** – The purpose of today's experiment was to prepare medium for the upcoming experiments and check the cell culture room for consumables, reagents, etc.

Experimental documentation

- **Materials / Reagents Used**
  - ➢ 50 mL Falcon tubes (x4)
  - ➢ 200 uL tips (x2)
  - ➢ 1.5 mL Eppendorf tubes (x2)
  - ➢ 5 mL stripettes (x3)
  - ➢ 10 mL stripettes (x2)
  - ➢ 5 mL syringe (x1)
  - ➢ filters (x2)
  - ➢ FBS (12,5 mL)
  - ➢ pen/strep (1 mL)
  - ➢ DMEM (87 mL)
- **Method / Protocol Reference**
  - ➢ In-house protocol for medium preparation
- **Sample / Cell Line**
  - ➢ Cell line: A-549

Observations & Results

- Two 50 mL Falcons of full medium were made with the following formulation
  - ❖ DMEM, 15 % FBS, 1% pen/strep
  - ❖ DMEM, 10 % FBS, 1% pen/strep
- A small aliquot of 200 uL was taken from each Falcon after the formulation and was transferred into a 1,5 mL tube to check for any contamination 24 hours after being stored in the incubator.

AUGUST 31ST 2026

Overview

- **Date** – Monday, August 31st 2026
- **Time (Start–End)** – 9:40 - 13:30
- **Name / Initials** – Erriketti Moumka (EM)
- **Shadowing** - Alexandros Vlachos, Anna Filindri
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Thawing of A-549 cell line
- **Objective** – The purpose of this day's experiment was to thaw the A-549 cell line to start the cell culture process.

Experimental documentation

- **Materials / Reagents Used**
  - ➢ T-25 flask (x1)
  - ➢ stripettes 5 mL (x4)
  - ➢ stripettes 10 mL (x3)
  - ➢ DMEM FBS 10% 1% p/s (24 mL)
  - ➢ DMEM FBS 15% 1% p/s (6 mL)
  - ➢ 15 ml Falcon (x4)
  - ➢ Trypan Blue (60 mL)
  - ➢ 10 µL tips (x20)
- **Method / Protocol Reference**
  - ➢ In-house protocol for cell thawing
- **Sample / Cell Line**
  - ➢ cell line: A-549

Observations & Results

- **Observations / Notes**
  - Before thawing, both complete media we prepared on Saturday were assessed with Trypan Blue staining under the microscope.
  - Two cryovials were thawed, which had both been prepared on 09/05/2026.
  - The pellet of cells after the centrifuge step was present in both Falcons, indicating that the thawing step was done correctly.
  - After resuspending the pellets in fresh DMEM with 15% FBS (6 mL total final volume), the flask containing cells was observed, and no abnormalities were noted.
- **Result Summary**
  - The timeframe in which the entirety of the cryovial volume was transferred to the diluent medium was about 3 minutes for the first cryovial, while it took 3 and a half minutes for the second one.
  - The cell thawing process took around 20 minutes in total for each cryovial.
  - The cells were observed, and no abnormalities were noted.

SEPTEMBER 1ST 2026

Overview

- **Date** – Tuesday, September 1st 2026
- **Time (Start–End)** – 10:40-11:20 & 13:30-13:40
- **Name / Initials** – Erriketti Moumka (EM)
- **Shadowing** – Alexandros Vlachos, Anna Filindri, Maria Loizou, Taxiarchis Kosmas
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Observation of A-549 cells
- **Objective** – The purpose of this day's experiment was to observe the A-549 cells to assess their morphology and decide whether or not they need a change of medium.

Experimental documentation

- **Materials / Reagents Used**

-

Observations & Results

- **Observations / Notes**
    - The color of the medium was changed to a paler pink, indicating that the cells had begun to metabolise.
    - Some cells had formed a monolayer, but a lot of the cells had still not settled and were observed in multiple planes.
    - Some cells with abnormal morphology were observed.
    - The cells were observed in two timepoints: during the first observation the confluency of the adherent cells was estimated at about 30-35%, while at the second observation, more cells had settled and the confluency estimate eas slightly higher at 35-40%.
- **Result Summary**
    - After the observation, we decided not to change the medium of the flask.

SEPTEMBER 2ND 2026

Overview

- **Date** – Wednesday, September 1st 2026
- **Time (Start–End)** – 10:40 - 11:10
- **Name / Initials** – Erriketti Moumka (EM)
- **Shadowing** – Alexandros Vlachos, Anna Filindri, Maria Loizou
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Observation of A-549 cells
- **Objective** – The purpose of this day's experiment was to observe the A-549 cells to assess their morphology and decide whether or not they need a change of medium.

Experimental documentation

- **Materials / Reagents Used**

-

Observations & Results

- **Observations / Notes**
    - The color of the medium was changed to a paler pink with a slight orange tint, indicating that the cells had begun to metabolise.
    - Cell density of the monolayer was higher than yesterday at around 40-45%, but a lot of the cells had still not settled and were observed in multiple planes.
    - Some cells with abnormal morphology were observed.
- **Result Summary**
    - After the observation, we decided not to change the medium of the flask.

SEPTEMBER 3RD 2026

Overview

- **Date** – Thursday September 3rd 2026
- **Time (Start–End)** – 10:50-14:40
- **Name / Initials** – Erriketti Moumka (EM)
- **Shadowing** - Margarita Papastergiou, Alexandros Vlachos, Anna Filindri, Maria Loizou
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Observation of A-549 cells and medium change
- **Objective** – The purpose of this day's experiment was to observe the A-549 cells in the T-25 cell culture flask, and to change the medium. We also performed heat inactivation and aliquoting of FBS (MP).

Experimental documentation

- **Materials / Reagents Used**
  - ➢ 15 mL Falcon tube (x1)
  - ➢ 50 mL Falcon tube (x10)
  - ➢ 5 mL stripettes (x4)
  - ➢ 10 mL stripettes (x1)
  - ➢ DMEM FBS 15% 1% P/S (6 mL)

Observations & Results

- **Observations / Notes**
  - The color of the medium had changed to a paler pink-orange, indicating that the cells had begun to metabolise.
  - The surface area the cells had taken up was around 50% in the T-25 flask, while a large number of cells had still not managed to attach.
  - Residual medium volume was 6,1 mL in the T-25 flask, a number explained by the residual volume not removed following the centrifugation step of the thawing process.
- **Result Summary**
  - The spent medium with any cells still not attached was collected and centrifuged. The presence of a not well-defined pellet confirmed the harvest of the suspended cells, which were resuspended in 3 mL of fresh DMEM FBS 15% medium, and returned to the cell culture flask.
  - Before centrifugation of the collected medium, 3 mL of fresh DMEM FBS 15% medium was placed in the flask, and the flask was returned to the incubator during waiting times.
  - Overall, the cells were observed and the medium was changed with 6 mL total fresh complete DMEM FBS 15% medium.
  - A new bottle of FBS that was thawed overnight was heat inactivated for 30 minutes in a 56 C waterbath, and made into 10 50 mL aliquots, which are stored in a -20 C freezer.

SEPTEMBER 4TH 2026

Overview

- **Date** – Friday, September 4th 2026
- **Time (Start–End)** – 13:40 - 14:00
- **Name / Initials** – Erriketti Moumka (EM)
- **Shadowing** – Alexandros Vlachos, Anna Filindri, Maria Loizou
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Observation of A-549 cells
- **Objective** – The purpose of this day's experiment was to observe the A-549 cells to assess their morphology and decide whether or not they need a change of medium.

Experimental documentation

- **Materials / Reagents Used**

-

Observations & Results

- **Observations / Notes**
    - The color of the medium was changed to a paler pink with a slight orange tint, indicating that the cells had begun to metabolise.
    - Cell density of the monolayer was higher than yesterday at around 50%, but a lot of the cells had still not settled and were observed in multiple planes.
    - Some cells with abnormal morphology were observed.
- **Result Summary**
    - After the observation, we decided not to change the medium of the flask.

SEPTEMBER 5TH 2026

Overview

- **Date** – Saturday, September 5th 2026
- **Time (Start–End)** – 10:00-11:00
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Erriketti Moumka, Alexandros Vlachos, Anna Filindri
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Observation of A-549 cells and medium change
- **Objective** – The purpose of this day's experiment was to observe the A-549 cells in the T-25 cell culture flask, and to change the medium.

Experimental documentation

- **Materials / Reagents Used**
  - ➢ 15 mL Falcon tube (x1)
  - ➢ 5 mL stripettes (x3)
  - ➢ DMEM FBS 15% 1% P/S (5,5 mL)

Observations & Results

Observations / Notes

- ❖ **Medium color:** On today's observation, the color of the culture medium had shifted to a slightly more orange hue. The change was subtle but more noticeable in person than in the photograph.
- ❖ **Floating cells:** Floating (suspended) cells were still present today.
- ❖ **Confluence:** Estimated at approximately 60%, maintaining the same distribution pattern as before: sparser near the edges of the flask and slightly denser toward the center.

Medium change performed:

- Final volume: 6 mL
- Composition: DMEM, 15% FBS, 1% P/S
- Of this volume: 0.5 mL from the cell suspension + 5.5 mL fresh medium



Fig. x: Picture of T-25 right after being taken out of the incubator

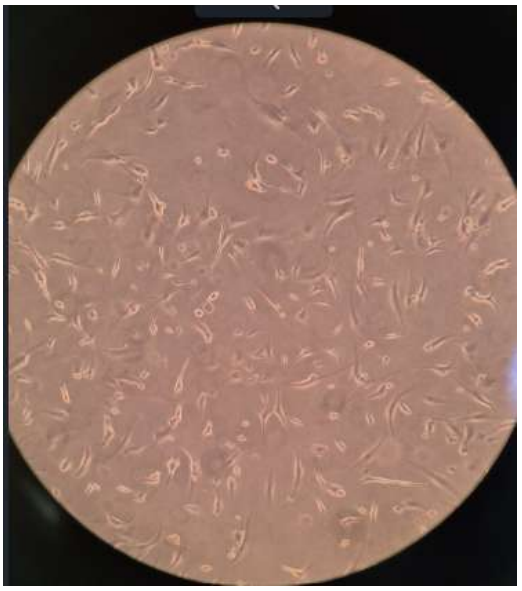


Fig. x: T-25 flask confluency, before change of medium (5x focus)

SEPTEMBER 6TH 2026

Overview

- **Date** – Sunday, September 6th 2026
- **Time (Start–End)** – 9:30 - 9:50
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** – Erriketti Moumka, Alexandros Vlachos, Anna Filindri, Maria Loizou
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Observation of A-549 cells
- **Objective** – The purpose of this day's experiment was to observe the A-549 cells to assess their morphology and decide whether or not they need a change of medium.

Experimental documentation

- **Materials / Reagents Used**

-

Observations & Results

Observations / Notes

- **Medium color:** The color of the medium had changed minimally since the last observation.
- **Floating cells:** The number of floating (suspended) cells had decreased significantly and noticeably.
- **Confluence:** Estimated at approximately 65%, with some areas showing higher confluence (the reported percentage reflects the representative confluence across the flask).



Fig. x: T-25 flask right after being taken out of the incubator

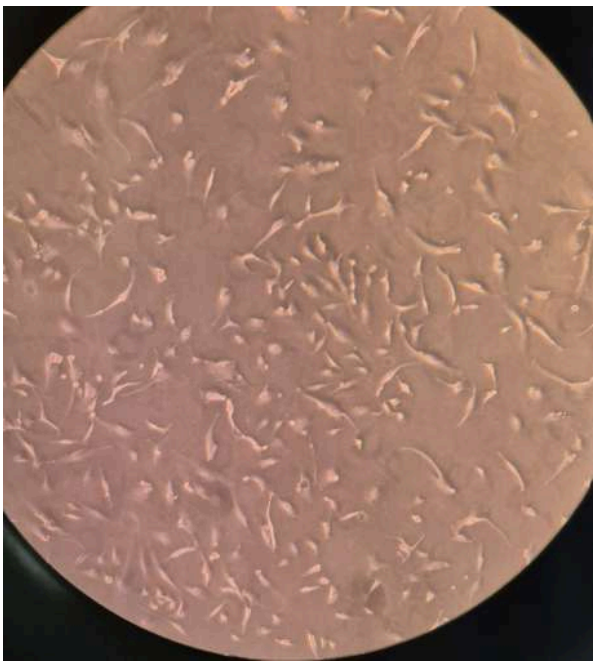


Fig. x: T-25 flask confluency (5x focus)

SEPTEMBER 7TH 2026

Overview

- **Date** – Monday, September 7th 2026
- **Time (Start–End)** – 9:10- 9:40
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Erriketti Moumka, Alexandros Vlachos, Anna Filindri
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Observation of A-549 cells and medium change
- **Objective** – The purpose of this day's experiment was to observe the A-549 cells in the T-25 cell culture flask, and to change the medium.

Experimental documentation

- **Materials / Reagents Used**
  - ➢ 30 mL Falcon tube (x1)
  - ➢ 5 mL stripettes (x2)
  - ➢ DMEM FBS 15% 1% P/S (5,5 mL)

Observations & Results

Observations / Notes

- **Medium color:** The color of the medium had changed to a slightly more orange hue.
- **Floating cells:** Floating (suspended) cells had almost disappeared, with only very few still present.
- **Confluence:** Estimated to be close to 70–75%.

Medium change performed:

- As usual, 0.5 mL of the old medium was retained
- 5.5 mL of fresh medium was added (DMEM, 15% FBS, 1% P/S)



Fig. x: T-25 flask right after being taken out of the incubator

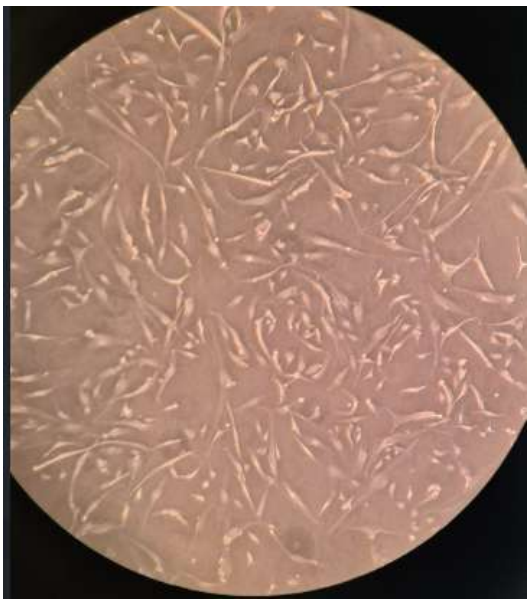


Fig. x: T-25 flask confluency (5x focus)

SEPTEMBER 8TH 2026

Overview

- **Date** – Tuesday, September 8th 2026
- **Time (Start–End)** – 9:10- 9:40
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Erriketti Moumka, Alexandros Vlachos, Anna Filindri
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Observation of A-549 cells and medium change
- **Objective** – The purpose of this day's experiment was to observe the A-549 cells in the T-25 cell culture flask, and to change the medium.

Experimental documentation

- **Materials / Reagents Used**
  - ➢ 30 mL Falcon tube (x1)
  - ➢ 5 mL stripettes (x2)
  - ➢ DMEM FBS 15% 1% P/S (5,5 mL)

Observations & Results

Observations / Notes

- **Flask observed:** T25 flask
- **Medium color:** The color of the medium had changed minimally since the previous day.
- **Floating cells:** The number of floating (suspended) cells appeared to have increased compared to yesterday.
- **Confluence:** Estimated to be close to 75%, as there was no major change compared to yesterday.

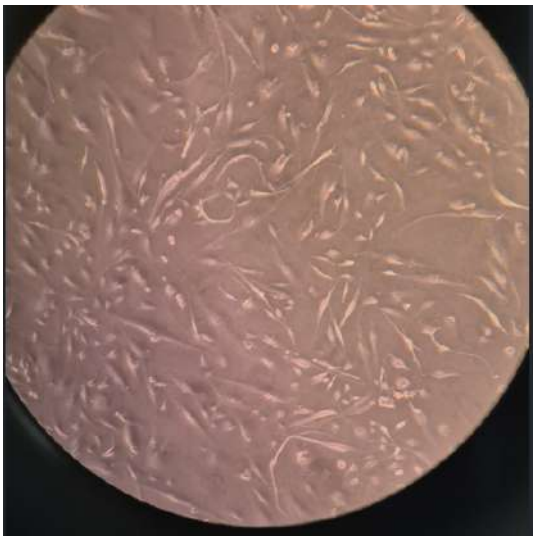
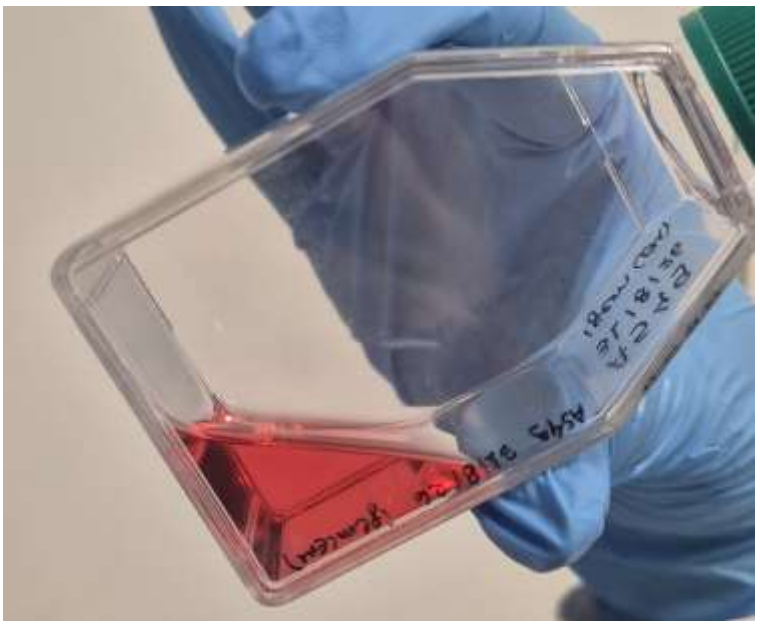


Fig. X: T-25 flask, right after being removed from the incubator.



T-25 flask - color of medium

SEPTEMBER 9TH 2026

Overview

- **Date** – Wednesday, September 9th 2026
- **Time (Start–End)** – 9:20- 11:00
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Erriketti Moumka, Alexandros Vlachos, Taxiarchis Kosmas
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Observation of A-549 cells, cryovial making
- **Objective** – The purpose of this day's experiment was to observe the A-549 cells in the T-25 cell culture flask, and to make one cryovial.

Experimental documentation

- **Materials / Reagents Used**
  - ➢ 30 mL Falcon tube (x1)
  - ➢ 5 mL stripettes (x10)
  - ➢ Cryovial with white cap (x1)
  - ➢ 15 mL Falcon (x2)
  - ➢ PBS 1X (2 mL)
  - ➢ DMEM FBS 15% 1% P/S (6 mL)
  - ➢ DMEM FBS 10% 1% P/S (2 mL)
  - ➢ Trypsin-EDTA (1 mL)
  - ➢ Freezing medium (1 mL)

Observations & Results

Observations / Notes

- The medium turned more orange, indicating the cells had metabolized.
- The confluency was around 80 % => we decided to make one cryovial with ⅓ of the flask volume and recultured the other ⅔ of the flask volume.

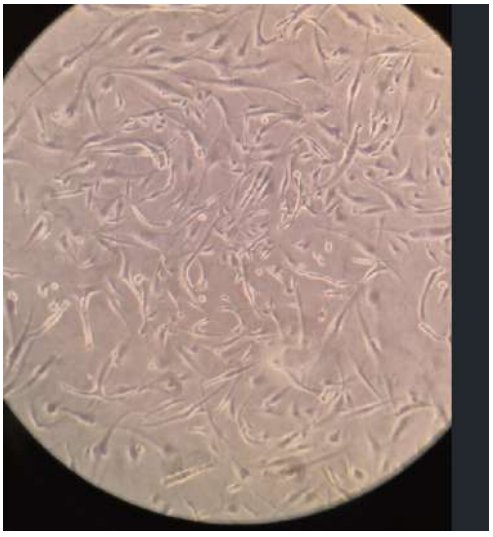


Fig. X: T-25 flask, right after being removed from the incubator.

- The final volume of the flask after the reculture process was 6 mL.
- After the reculture, the cells were observed, and no abnormalities were noted.

SEPTEMBER 10TH 2026

Overview

- **Date** – Thursday, September 10th 2026
- **Time (Start–End)** – 9:10- 9:30
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Erriketti Moumka, Alexandros Vlachos, Taxiarchis Kosmas
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Observation of A-549 cells
- **Objective** – The purpose of this day's experiment was to observe the A-549 cells in the T-25 cell culture flask.

Experimental documentation

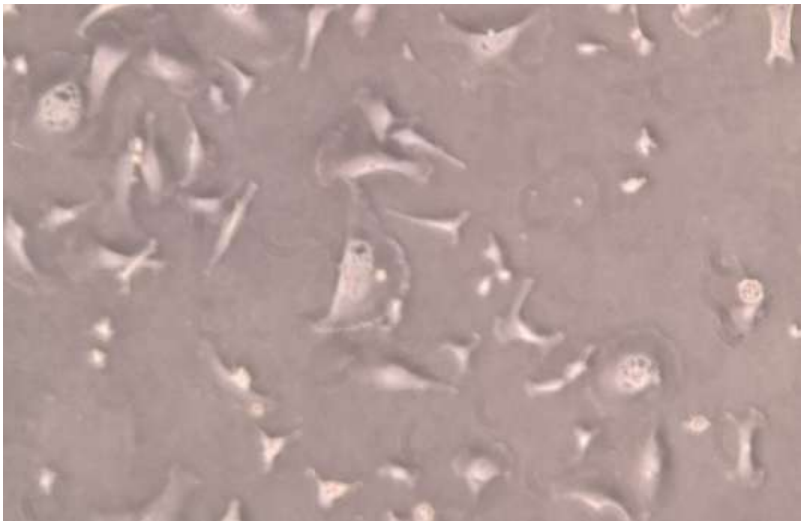
- **Materials / Reagents Used**

-

Observations & Results

Observations / Notes

- After observing the cells, a large percentage of the cells showed abnormalities, as shown in the following picture.



- The confluency was around 40%.
- The medium color was slightly more orange than the previous day.

SEPTEMBER 11TH 2026

Overview

- **Date** – Friday, September 11th, 2026
- **Time (Start–End)** – 9:15- 9:25
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Erriketti Moumka, Alexandros Vlachos, Anna Filindri
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Observation of A-549 cells and medium change
- **Objective** – The purpose of this day's experiment was to observe the A-549 cells in the T-25 cell culture flask, and to change the medium.

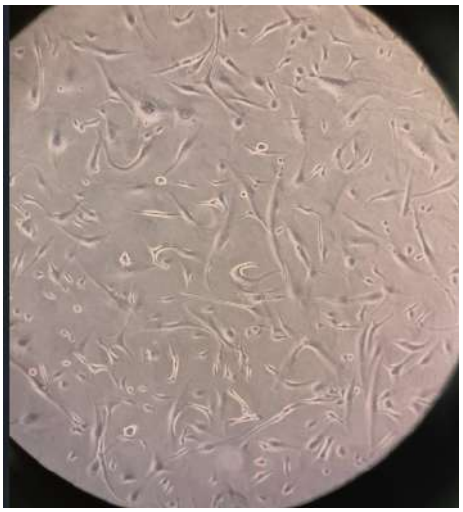
Experimental documentation

- **Materials / Reagents Used**
  - ➢ 15 mL Falcon tube (x1)
  - ➢ 5 mL stripettes (x2)
  - ➢ DMEM FBS 15% 1% P/S (5,5 mL)

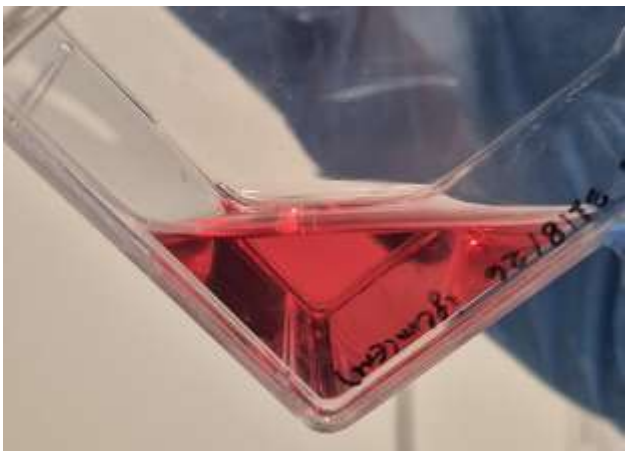
Observations & Results

Observations / Notes

- **Flask observed:** T25 flask
- **Medium color:** The color of the medium had changed minimally since the previous day.
- **Confluence:** Estimated to be close to 55%, as there was no major change compared to yesterday.



T-25 flask immediately after removal from the incubator



Color of medium immediately after removal from the incubator.

SEPTEMBER 12TH 2026

Overview

- **Date** – Saturday, September 12th, 2026
- **Time (Start–End)** – 9:10- 9:25
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Erriketti Moumka, Alexandros Vlachos, Anna Filindri
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Observation of A-549 cells
- **Objective** – The purpose of this day's experiment was to observe the A-549 cells in the T-25 cell culture flask.

Experimental documentation

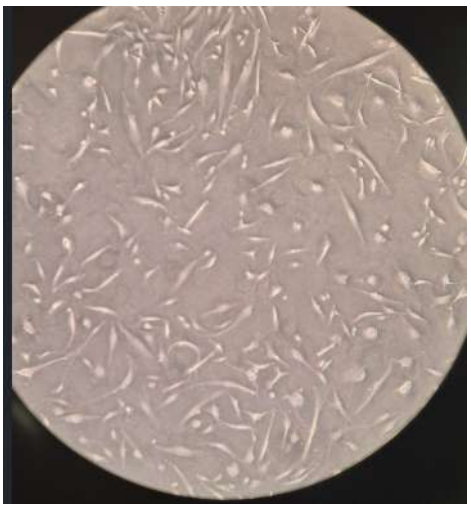
- **Materials / Reagents Used**

-

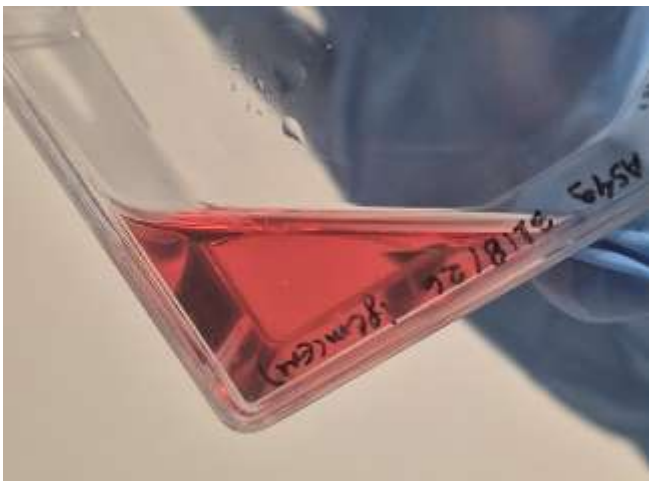
Observations & Results

Observations / Notes

- The confluency was around 60%.
- The medium color was slightly more orange than the previous day.
- No abnormalities were noted.



T-25 flask immediately after removal from the incubator



Color of medium immediately after removal from the incubator.

SEPTEMBER 13TH 2026

Overview

- **Date** – Sunday, September 13th 2026
- **Time (Start–End)** – 9:15- 9:30
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Erriketti Moumka, Alexandros Vlachos, Anna Filindri
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Observation of A-549 cells and medium change
- **Objective** – The purpose of this day's experiment was to observe the A-549 cells in the T-25 cell culture flask, and to change the medium.

Experimental documentation

- **Materials / Reagents Used**
  - ➢ 15 mL Falcon tube (x1)
  - ➢ 5 mL stripettes (x2)
  - ➢ DMEM FBS 15% 1% P/S (5,5 mL)

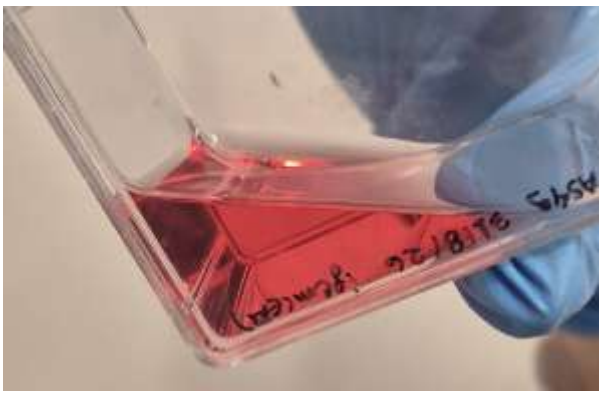
Observations & Results

Observations / Notes

- **Flask observed:** T25 flask
- **Medium color:** The color of the medium had changed minimally since the previous day.
- **Confluence:** Estimated to be close to 60%, as there was no major change compared to yesterday.



T-25 flask immediately after removal from the incubator



Color of medium after removal from the incubator

SEPTEMBER 14TH 2026

Overview

- **Date** – Monday, September 14th 2026
- **Time (Start–End)** – 9:50- 10:50
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Erriketti Moumka, Taxiarchis Kosmas
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Observation of A-549 cells
- **Objective** – The purpose of this day's experiment was to observe the A-549 cells in the T-25 cell culture flask.

Experimental documentation

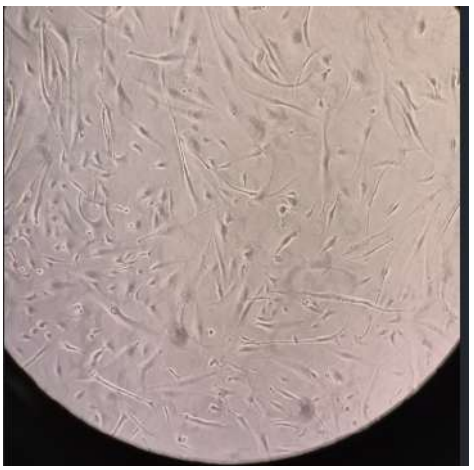
- **Materials / Reagents Used**

-

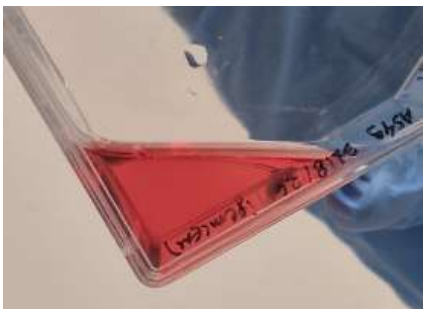
Observations & Results

Observations / Notes

- The confluency was around 70%.
- The medium color was slightly more orange than the previous day.
- No abnormalities were noted.



T-25 flask immediately after removal from the incubator.



Color of medium immediately after removal from the incubator.

SEPTEMBER 15TH 2026

Overview

- **Date** – Tuesday, September 15th 2026
- **Time (Start–End)** – 9:40- 11:55
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Erriketti Moumka, Taxiarchis Kosmas, Alexandros Vlachos
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Observation of A-549 cells, Split of A-549 cells, Seeding of cells for scraping practice
- **Objective** – The purpose of this day's experiment was to observe the A-549 cells in the T-25 cell culture flask and split them into a second T-75 flask.

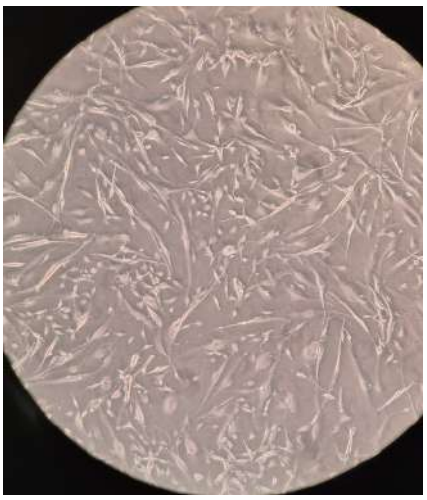
Experimental documentation

- **Materials / Reagents Used**
  - ❖ T-75 flask (x1)
  - ❖ 15 mL Falcons (x3)
  - ❖ 1,5 mL tubes (x2)
  - ❖ 5 mL stripettes (x8)
  - ❖ 10 mL stripettes (x1)
  - ❖ PBS 1x (2 mL)
  - ❖ Trypsin-EDTA 0,05% (1 mL)
  - ❖ Trypan Blue (30 uL)
  - ❖ DMEM, 10% FBS, 1% p/s (2 mL)
  - ❖ DMEM, 15% FBS, 1% p/s (16 mL)
  - ❖ 200 uL tips (x5)
  - ❖ 96-well plate (x1)
  - ❖ 20 uL tips (x3)

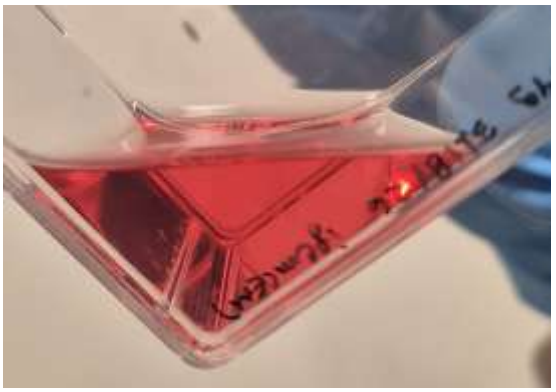
Observations & Results

Observations / Notes

- The T-25 flask prior to any experimental procedure was observed and the confluency was estimated around 90%.



T-25 flask immediately after removal from the incubator.



Color of medium immediately after removal from the incubator.

- The spit ratio of the cell suspension was:
    - ⅓ of the cell suspension volume -> T-25 (final flask volume: 6 mL)
    - ⅔ of the cell suspension volume -> T-75(final flask volume: 10 mL)

Seeding

- The reason behind this seeding process was because during the N-3a experiments we experienced difficulties during the cell harvesting step.
- After consulting Dr. Nikolakaki, we decided to seed 2 wells in a 96-well plate with 20k cells/ well, and perform the scraping step in two different timepoints.
- The seeding procedure went well and no abnormalities were noted.

SEPTEMBER 16TH 2026

Overview

- **Date** – Wednesday, September 16th 2026
- **Time (Start–End)** – 10:55- 11:15
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Erriketti Moumka, Anna Filindri, Alexandros Vlachos
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Observation of A-549 cells
- **Objective** – The purpose of this day's experiment was to observe the A-549 cells in the T-25 and T-75 cell culture flasks.

Experimental documentation

- **Materials / Reagents Used**

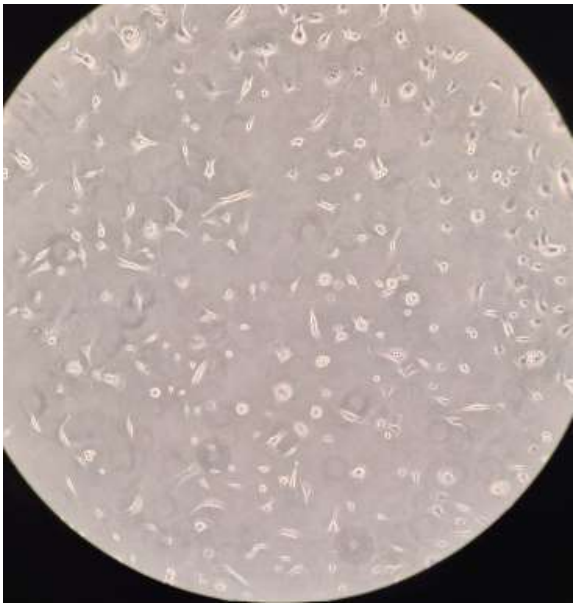
-

Observations & Results

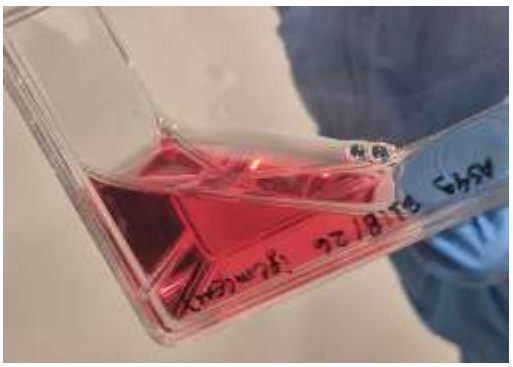
Observations / Notes

T-25 flask

- The confluency was around 50%.
- The medium color was slightly more orange than the previous day.
- No abnormalities were noted.



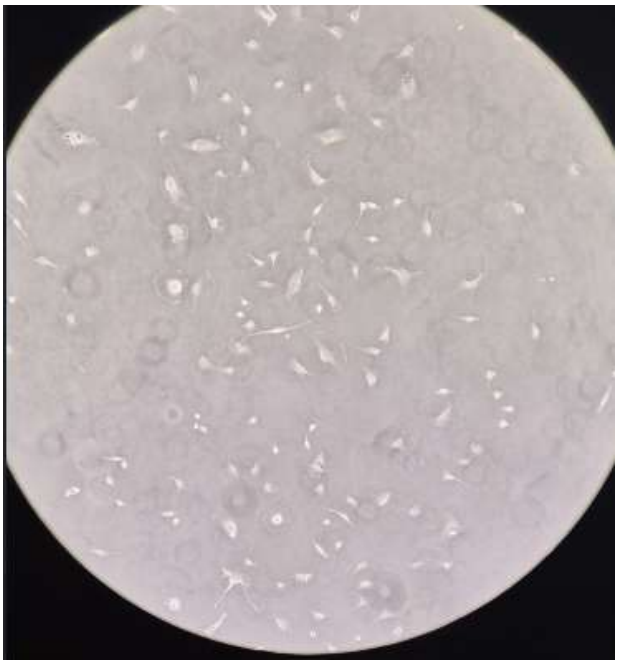
T-25 flask immediately after removal from the incubator.



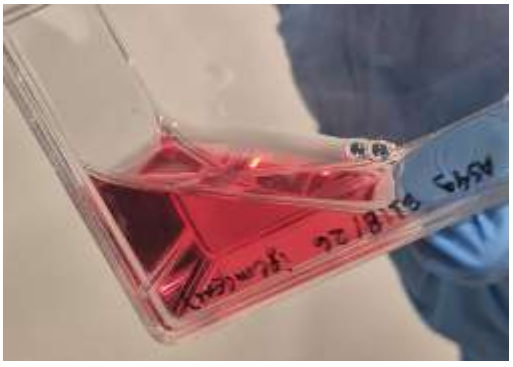
Color of medium immediately after removal from the incubator.

T-75 flask

- The confluency was around 30%.
- The medium color was slightly more orange than the previous day.
- No abnormalities were noted.



T-75 flask immediately after removal from the incubator.



Color of medium immediately after removal from the incubator.

SEPTEMBER 17TH 2026

Overview

- **Date** – Thursday, September 17th 2026
- **Time (Start–End)** – 9:45- 11:00
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Erriketti Moumka, Alexandros Vlachos, Anna Filindri
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Observation of A-549 cells and medium change
- **Objective** – The purpose of this day's experiment was to observe the A-549 cells in the T-25 and T-75 cell culture flask, and to change the medium.

Experimental documentation

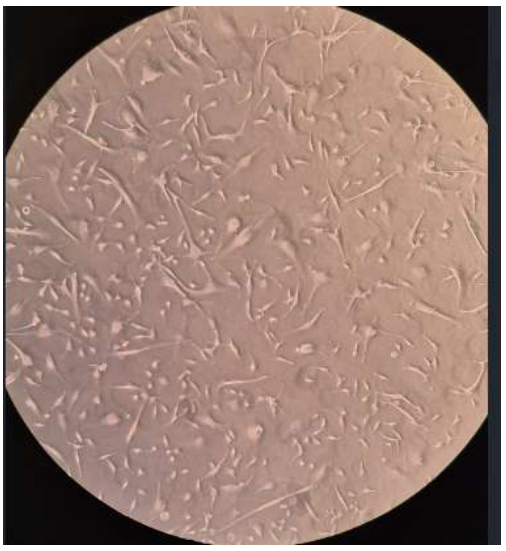
- **Materials / Reagents Used**
  - ➢ 50 mL Falcon tube (x1)
  - ➢ 5 mL stripettes (x4)
  - ➢ DMEM FBS 15% 1% P/S (14,5 mL)
  - ➢ PBS 1x (50 uL)
  - ➢ Trypan Blue (30 uL)
  - ➢ 200 uL tips (x3)
  - ➢ 20 uL tips (x3)

Observations & Results

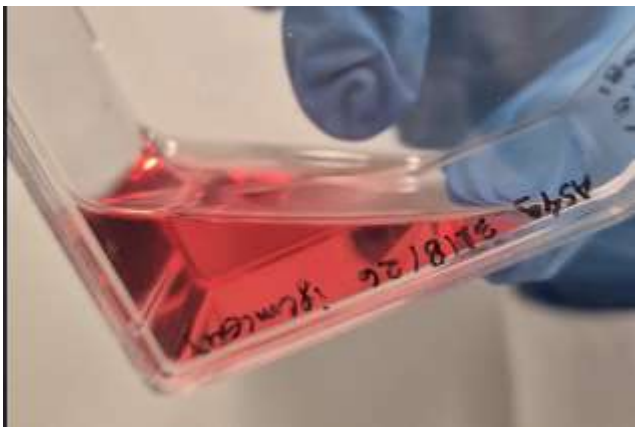
Observations / Notes

T-25 flask

- The confluency was around 55%.
- The medium color was slightly more orange than the previous day.
- No abnormalities were noted.



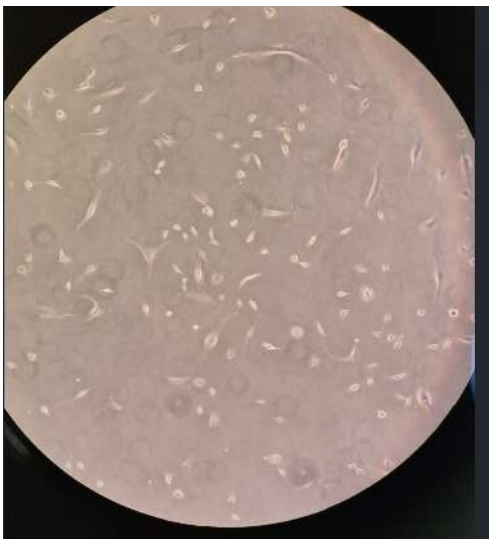
T-25 flask immediately after removal from the incubator.



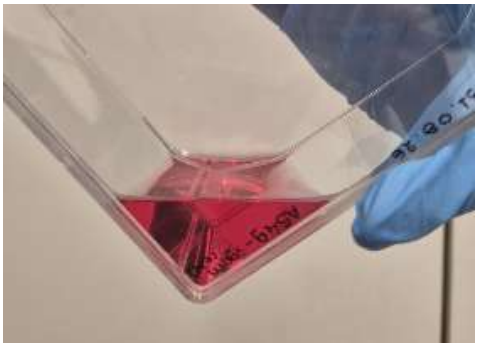
Color of medium immediately after removal from the incubator.

T-75 flask

- The confluency was around 30%.
- The medium color was slightly more orange than the previous day.
- No abnormalities were noted.



T-75 flask immediately after removal from the incubator.



Color of medium immediately after removal from the incubator.

SEPTEMBER 18TH 2026

Overview

- **Date** – Friday, September 18th 2026
- **Time (Start–End)** – 10:10- 10:30
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Alexandros Vlachos, Taxiarchis Kosmas, Anna Filindri
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Observation of A-549 cells
- **Objective** – The purpose of this day's experiment was to observe the A-549 cells in the T-25 cell culture flask and T-75 cell culture flask.

Experimental documentation

- **Materials / Reagents Used**

-

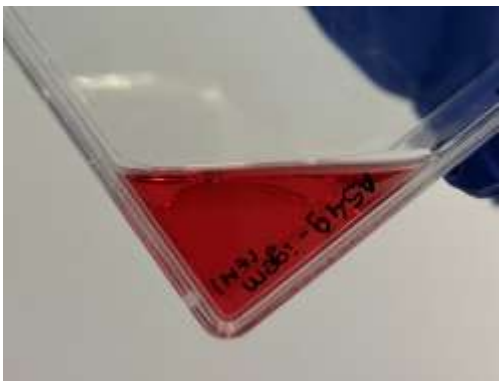
Observations & Results

Observations / Notes

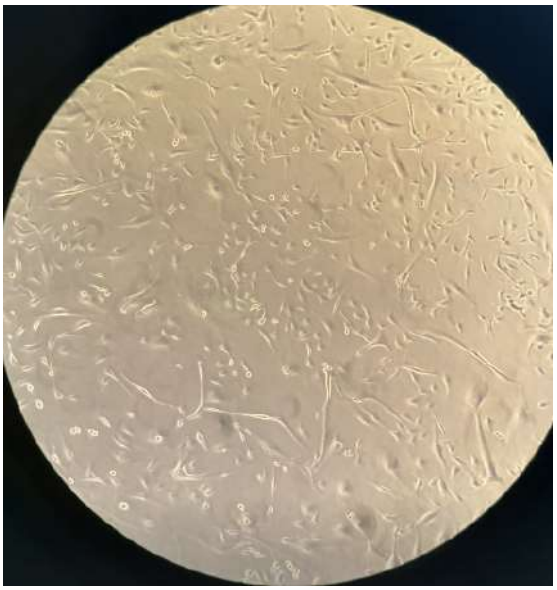
- The confluency was around:
  - ❖ 60% at the T-25 cell culture flask.
  - ❖ 35% at the T-75 cell culture flask.
- The medium color was slightly more orange than the previous day.
- No abnormalities were noted.



T-75 flask immediately after removal from the incubator.



Color of medium immediately after removal from the incubator - T-75 flask.



T-25 flask immediately after removal from the incubator.

SEPTEMBER 19TH 2026

Overview

- **Date** – Saturday, September 19th, 2026
- **Time (Start–End)** – 8:20- 8:48
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Taxiarchis Kosmas, Alexandros Vlachos, Anna Filindri
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Observation of A-549 cells and medium change
- **Objective** – The purpose of this day's experiment was to observe the A-549 cells in the T-25 and T-75 cell culture flask.

Experimental documentation

- **Materials / Reagents Used**
  -

Observations & Results

T-25 flask

- The confluency was around 65%.
- The medium color was slightly more orange than the previous day.
- No abnormalities were noted.



T-25 flask immediately after removal from the incubator.

T-75 flask

- The confluency was around 40%.
- The medium color was slightly more orange than the previous day.
- No abnormalities were noted.



T-75 flask immediately after removal from the incubator.

SEPTEMBER 20TH 2026

Overview

- **Date** – Sunday, September 20th, 2026
- **Time (Start–End)** – 9:30- 12:20
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Erriketti Theodora Moumka, Alexandros Vlachos, Anna Filindri
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Observation of A-549 cells and medium change
- **Objective** – The purpose of this day's experiment was to observe the A-549 cells in the T-25 and T-75 cell culture flask, and to split the cells in the T25 flask and change the medium.

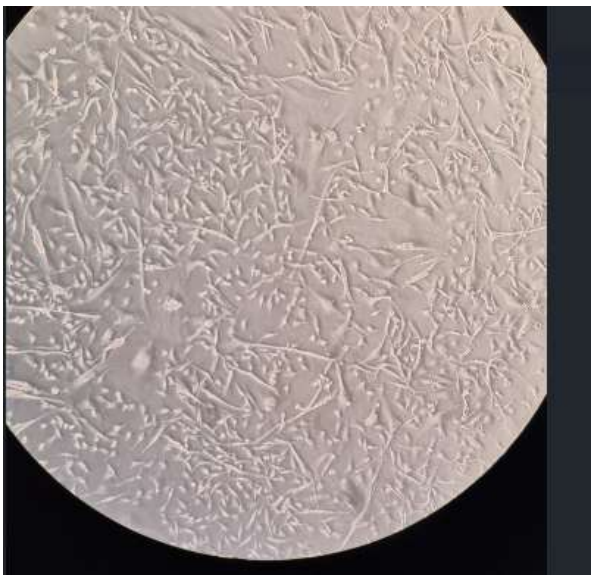
Experimental documentation

- **Materials / Reagents Used**
  - T-75 flask
  - DMEM, 15% FBS, 1% p/s (26 mL)
  - 10 mL stripettes (x2)
  - 5 mL stripettes (x10)
  - 15 mL Falcons (x2)
  - PBS (2mL)
  - Trypsin-EDTA (1 mL)
  - DMEM, 10% FBS, 1% p/s (2 mL)
  - 1,5 mL tube (x2)
  - Trypan Blue (30 uL)
  - 20 uL tips (x3)
  - 50 mL Falcons (x2)
  - DMEM (42 mL)
  - FBS (7,5 mL)
  - p/s (0,5 mL)
  - filter (x1)
  - syringe (x1)

Observations & Results

T-25 flask

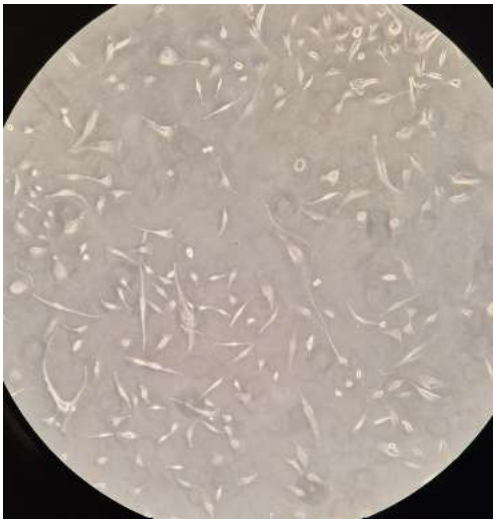
- The confluency was around 82% => split
  - ⅓ of the volume -> T-25 flask
  - ⅔ of the volume -> T-75 flask
- The medium color was slightly more orange than the previous day.
- No abnormalities were noted.



T-25 flask immediately after removal from the incubator.

T-75 flask

- The confluency was around 35%
- The medium color was slightly more orange than the previous day.
- No abnormalities were noted.



T-75 flask immediately after removal from the incubator.

SEPTEMBER 21ST 2026

Overview

- **Date** – Monday, September 21st, 2026
- **Time (Start–End)** – 10:30- 11:00
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Erriketti Theodora Moumka
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Observation of A-549 cells and medium change
- **Objective** – The purpose of this day's experiment was to observe the A-549 cells in the T-25 and T-75 cell culture flasks.

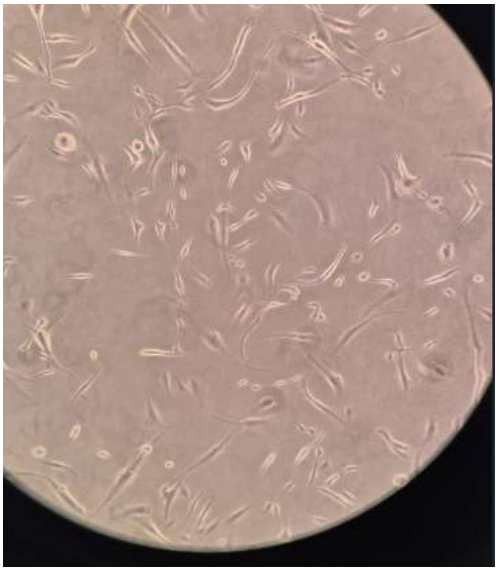
Experimental documentation

- **Materials / Reagents Used**
  -

Observations & Results

T-75 flask (OLD)

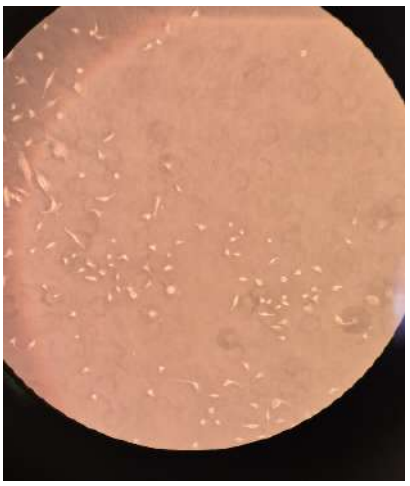
- The confluency was around 45%.
- The medium color was slightly more orange than the previous day.
- No abnormalities were noted.



T-75 flask immediately after removal from the incubator.

T-75 flask (NEW)

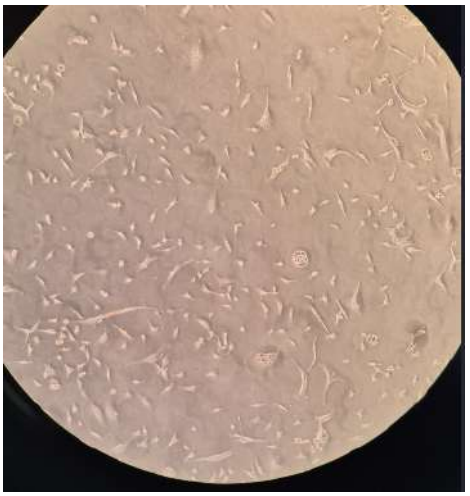
- The confluency was around 40%.
- The medium color was slightly more orange than the previous day.
- No abnormalities were noted.



T-75 flask immediately after removal from the incubator.

T-25 flask

- The confluency was around 35%.
- The medium color was slightly more orange than the previous day.
- No abnormalities were noted.



T-25 flask immediately after removal from the incubator.

SEPTEMBER 22ND 2026

Overview

- **Date** – Tuesday, September 22nd, 2026
- **Time (Start–End)** – 9:30- 10:30
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Alexandros Vlachos, Erriketti Theodora Moumka
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Observation of A-549 cells
- **Objective** – The purpose of this day's experiment was to observe the A-549 cells in the T-25 cell culture flask and T-75 cell culture flasks and change their medium, as well as make more medium.

Experimental documentation

- **Materials / Reagents Used**
  - DMEM, 15% FBS, 1% p/s (26 mL)
  - 10 mL stripettes (x7)
  - 5 mL stripettes (x3)
  - 50 mL Falcons (x2)
  - filter (x1)
  - syringe (x1)
  - DMEM (42 mL)
  - p/s (0,5 mL)
  - FBS (7,5 mL)
  - 200 uL tip (x1)
  - 1,5 mL tube (x1)

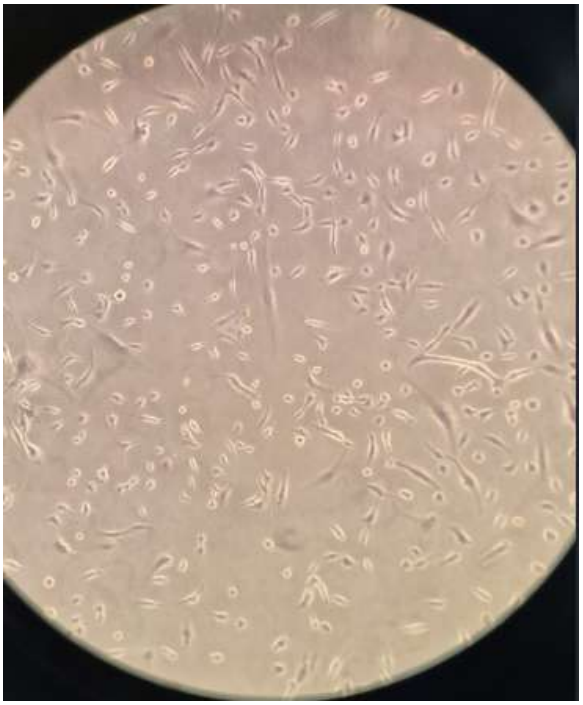
Observations & Results

Observations / Notes

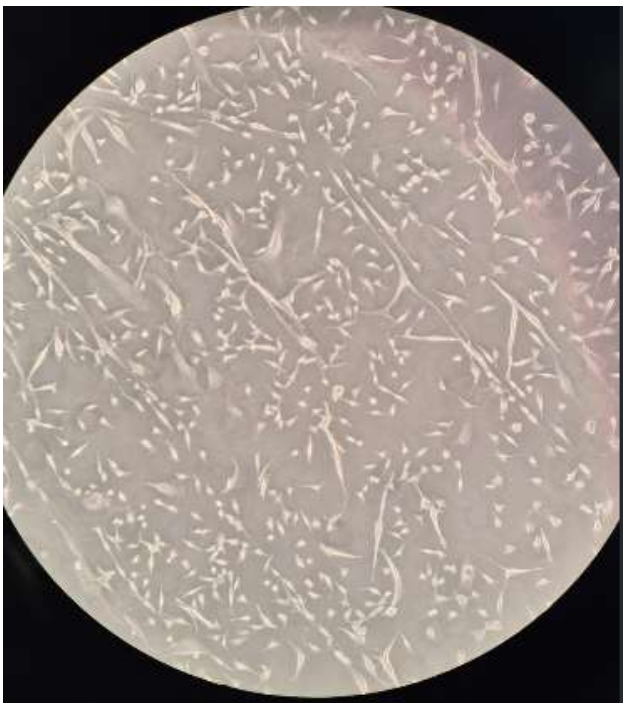
- The confluency, adjusted to account for large empty spots observed in both T75 flasks, was around:
  - T25: 60%
  - T75 old: 45%
  - T75 new: 30%
- The medium color was slightly more orange than the previous day.



T-75 (OLD) flask immediately after removal from the incubator.



T-75 (NEW) flask immediately after removal from the incubator.



T-25 flask immediately after removal from the incubator.

SEPTEMBER 23RD 2026

Overview

- **Date** – Wednesday, September 23rd, 2026
- **Time (Start–End)** – 8:30- 11:50
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Alexandros Vlachos, Anna Filindri
- **Project / Subproject** – Cellular effect of pan-KRAS PROTAC in A-549 cells
- **Experiment / Task Title** – Seeding of A-549 cells
- **Objective** – The purpose of this day's experiment was to seed the A-549 cells in 96-well plates for the upcoming experiment.

Experimental documentation

- **Materials / Reagents Used**
  - 96-well plate (x2)
  - 5 mL stripettes (x10)
  - 10 mL stripettes (x11)
  - PBS 1X (7 mL)
  - Trypsin-EDTA (4 mL)
  - DMEM, 10% FBS, 1% p/s (8 mL)
  - DMEM, 15% FBS, 1% p/s (24,4 mL)
  - 1,5 mL tube (x1)
  - 50 mL Falcons (x3)
  - 15 mL Falcon (x1)
  - 200 uL tips (x12)
  - 1 mL tips (x6)
  - syringe (x1)
  - filter (x1)

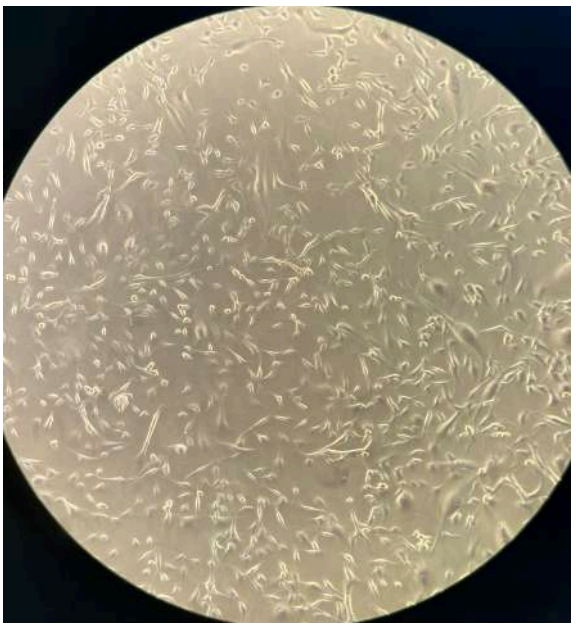
Observations & Results

- The T-25 flask was observed and the confluency was around 85%
- The old T-75 flask was observed and the confluency was around 60%
- Based on the calculations for the conditions, the needed number of cells was 1.840.000 cells
- Trypan Blue staining was performed for these two flasks:

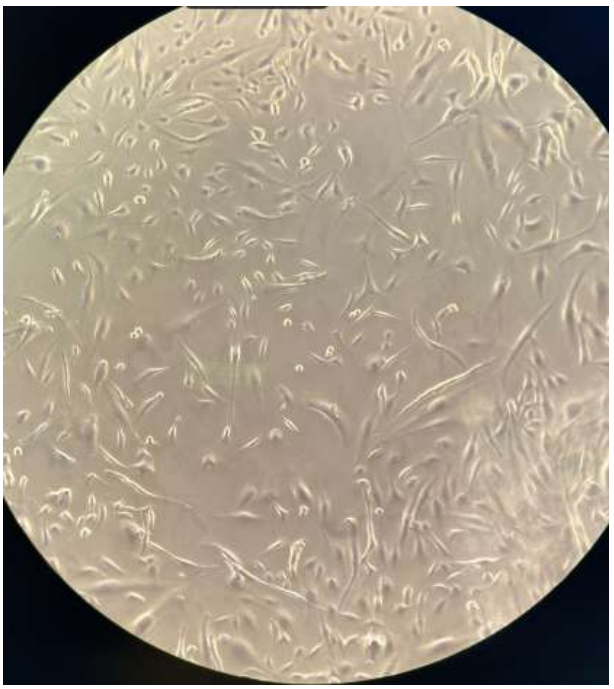
T25							
A549_T-25_MP	live	dead	Avg live	Avg dead	all	% Viability	cell/ml
5	11	1	7,75	1,25	9	86,11111111	**155000**
6	7	2					
7	6	1					
8	7	1					
						TOTAL C.N	465000

T75							
A549_T-25_MP	live	dead	Avg live	Avg dead	all	% Viability	cell/ml
5	11	1	9,25	0,5	9,75	94,87179487	**185000**
6	9	0					
7	7	1					
8	10	0					
						TOTAL C.N	1665000

- We used the entire volume of the T-75 flask and 1.13 mL from the T-25 flask to retain the total cell number for seeding.
- The rest of the T-25 flask volume was centrifuged and recultured in a total volume of 6 mLs.
- The pellet was resuspended in 18.3 mLs and 200 uL was seeded per well in a zig-zag motion, seeding 100 uL each time.
- After the seeding was completed, the plates were observed, and no abnormalities were noted.
    - ❖ The cells had taken up mostly the upper side of each well, with fewer taking up the lower side.



T-25 Flask immediately after being taken out of the incubator.



T-75 Flask immediately after being taken out of the incubator.

SEPTEMBER 23rd 2026

Overview

- **Date** – Wednesday, September 23rd, 2026
- **Time (Start–End)** – 8:30- 11:50
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Alexandros Vlachos, Anna Filindri
- **Project / Subproject** – Cellular effect of pan-KRAS PROTAC in A-549 cells
- **Experiment / Task Title** – Seeding of A-549 cells
- **Objective** – The purpose of this day's experiment was to seed the A-549 cells in 96-well plates for the upcoming experiment.

Experimental documentation

- **Materials / Reagents Used**
  - 96-well plate (x2)
  - 5 mL stripettes (x2)
  - 10 mL stripettes (x11)
  - PBS 1X (7 mL)
  - Trypsin-EDTA (4 mL)
  - DMEM, 10% FBS, 1% p/s (8 mL)
  - DMEM, 15% FBS, 1% p/s (38,7 mL)
  - 1,5 mL tube (x1)
  - 50 mL Falcons (x1)
  - 15 mL Falcon (x7)
  - 200 uL tips (x35)
  - 1 mL tips (x6)

Observations & Results

- The T-25 flask was observed and the confluency was around 85%
- The old T-75 flask was observed and the confluency was around 60%
- Based on the calculations for the conditions, the needed number of cells was 1.840.000 cells
- Trypan Blue staining was performed for these two flasks:

T25

A549_T-25_MP	live	dead	Avg live	Avg dead	all	% Viability	cell/ml
5	11	1	7,75	1,25	9	86,11111111	**155000**
6	7	2					
7	6	1					
8	7	1					
						TOTAL C.N	465000

T75							
A549_T-25_MP	live	dead	Avg live	Avg dead	all	% Viability	cell/ml
5	11	1	9,25	0,5	9,75	94,87179487	**185000**
6	9	0					
7	7	1					
8	10	0					
						TOTAL C.N	1665000

- We used the entire volume of the T-75 flask and 1.13 mL from the T-25 flask to retain the total cell number for seeding.
- The rest of the T-25 flask volume was centrifuged and recultured in a total volume of 6 mLs.
- The pellet was resuspended in 18.3 mLs and 200 uL was seeded per well in a zig-zag motion, seeding 100 uL each time.
- After the seeding was completed, the plates were observed, and no abnormalities were noted.
    - ❖ The cells had taken up mostly the upper side of each well, with fewer taking up the lower side.
- Before passaging, the A-549 cells in the culture flask appeared healthy under the microscope, with normal epithelial morphology, good adherence and approximately 85% confluency. No signs of contamination (turbidity, colour change of the medium or floating debris) were observed.
- After trypsinisation with Trypsin-EDTA, the cells detached completely within 4 minutes and formed a homogeneous single-cell suspension after gentle pipetting, with no visible clumps.
- The cell suspension was mixed between pipetting steps to keep the cells evenly distributed, and all wells received the same volume.
- Shortly after seeding, the cells were evenly spread across the wells under the microscope, with no obvious differences between wells. The plates were placed in the incubator at 37 °C and 5% CO_2 to allow attachment before treatment.
- No problems or deviations from the protocol occurred during the procedure.

SEPTEMBER 24TH 2026

Overview

- **Date** – Thursday, September 24th, 2026
- **Time (Start–End)** – 8:30- 11:50
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Alexandros Vlachos, Anna Filindri
- **Project / Subproject** – Cellular effect of pan-KRAS PROTAC in A-549 cells
- **Experiment / Task Title** – Treatment of A-549 cells
- **Objective** – The purpose of this day's experiment was to treat the A-549 cells with ACBI3.

Experimental documentation

- **Materials / Reagents Used**
  - 15 mL Falcons (x7)
  - 50 mL Falcon (x1)
  - 200 µL tips (x35)
  - 1 mL tips (x11)
  - 5 mL stripettes (x2)
  - DMEM, 15% FBS, 1% p/s (38,7 mL)
  - DMSO (2 µL)
  - ACBI3 (pan-KRAS PROTAC) (2 µL)
  - cis-ACBI3 (inactive pan-KRAS PROTAC) (2 µL)

Observations & Results

- Before treatment, the A-549 cells in both 96-well plates appeared healthy and well attached, with normal epithelial morphology and approximately 70% confluency. Cell density was similar across all wells, and no signs of contamination were observed.
- ACBI3 and cis-ACBI3 stock solutions were fully dissolved in DMSO, and no precipitation was visible after dilution in DMEM (10% FBS, 1% p/s) to the final working concentrations of 10 µM.
- The working master mixes were made based on the table below:

Condition	Compound	DMSO (µL)	Volume from 10 µM intermediate stock (µL)	Medium (µL)	Final DMSO%
Baseline control	DMSO only	400	-	3600	0,01%
10 nM ACBI3	PROTAC	-	4	3996	0,0001%
100 nM	PROTAC	-	40	3960	0,001%

ACBI3					
1 µM ACBI3	PROTAC	-	400	3600	0,01%
10 nM cis-ACBI3	inhibitor	-	4	3996	0,0001%
100 nM cis-ACBI3	inhibitor	-	40	3960	0,001%
1 µM cis-ACBI3	inhibitor	-	400	3600	0,01%

- The old medium was carefully removed from each well and replaced with 200 µL of the corresponding treatment medium, without disturbing the cell monolayer. Wells were treated according to the plate layout, with ACBI3, the inactive control cis-ACBI3, and the DMSO vehicle control in 9 technical replicates.
- Immediately after treatment, no morphological changes or cell detachment were observed in any condition, as expected at this early time point.
- The plates were returned to the incubator at 37 °C and 5% CO_2 for24 hours until the next step of the experiment.
- No problems or deviations from the protocol occurred during the procedure.

SEPTEMBER 25TH 2026

Overview

- **Date** – Friday, September 25th, 2026
- **Time (Start–End)** – 8:30- 11:50
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - -
- **Project / Subproject** – Cellular effect of pan-KRAS PROTAC in A-549 cells
- **Experiment / Task Title** –Cell Viability assay 24 hours post ACBI3 treatment
- **Objective** – The purpose of this day's experiment was to perform cell viability assays 24 hours post ACBI3 treatment

Experimental documentation

- **Materials / Reagents Used**
  - 50 mL Falcon (x1)
  - 10 µL tips (x21)
  - 200 µL tips (x33)
  - 1 mL tips (x28)
  - 1,5 mL tubes (x21)
  - 10 mL stripettes (x2)
  - Trypan Blue (210 µL)
  - PBS 1X (1,05 mL)
  - FBS (3,1 mL)
  - Trypsin-EDTA (1,05 mL)
  - DMEM, 15% FBS, 1% p/s (20 mL)

Observations & Results

- 24 hours after treatment, the cells were examined under the microscope before harvesting. Cells in the DMSO control wells appeared healthy and well attached, with normal epithelial morphology and approximately 60% confluency. In the ACBI3-treated wells, no visible changes were observed compared to the control, while the cis-ACBI3-treated wells looked similar to the DMSO control.
- The medium from each well was collected together with the PBS wash so that any floating (dead) cells were included in the count. The adherent cells detached completely after trypsinisation with Trypsin-EDTA and were resuspended into a homogeneous single-cell suspension.
- Each sample was mixed 1:1 with Trypan Blue and counted. Viable cells excluded the dye and appeared bright and round, while dead cells were stained blue.
- ACBI3 treatment resulted in no significant change in the number of viable cells and in viability compared to the DMSO control, whereas cis-ACBI3 showed a comparable result to the control, as expected for the inactive analogue.
- No problems or deviations from the protocol occurred during the procedure.

SEPTEMBER 26TH 2026

Overview

- **Date** – Saturday, September 26th, 2026
- **Time (Start–End)** – 8:30- 11:50
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Taxiarchis Kosmas
- **Project / Subproject** – Cellular effect of pan-KRAS PROTAC in A-549 cells
- **Experiment / Task Title** – Cell Viability assay 48 hours post ACBI3 treatment
- **Objective** – The purpose of this day's experiment was to perform cell viability assays 48 hours post ACBI3 treatment

Experimental documentation

- **Materials / Reagents Used**
  - 50 mL Falcon (x1)
  - 10 µL tips (x21)
  - 200 µL tips (x33)
  - 1 mL tips (x28)
  - 1,5 mL tubes (x21)
  - 10 mL stripettes (x2)
  - Trypan Blue (210 µL)
  - PBS 1X (1,05 mL)
  - FBS (3,1 mL)
  - Trypsin-EDTA (1,05 mL)
  - DMEM, 15% FBS, 1% p/s (20 mL)

Observations & Results

- At 48 hours post-treatment, the plates were checked under the microscope prior to harvesting. The DMSO control cells had continued to proliferate since the 24-hour time point, reaching approximately 70% confluency while maintaining a healthy, adherent epithelial morphology. Cells exposed to ACBI3 showed a lower cell density relative to the control, whereas cells exposed to cis-ACBI3 closely resembled the vehicle-treated cells.
- To make sure no dead cells were lost, the culture medium and the PBS wash were collected from each well before trypsinisation. Trypsin-EDTA detached the remaining adherent cells efficiently, and the samples were pipetted gently to obtain single-cell suspensions. Equal volumes of cell suspension and Trypan Blue were then mixed and counted, distinguishing unstained viable cells from blue-stained dead cells.
- Overall, ACBI3 reduced the number of viable cells compared to the vehicle control, while cis-ACBI3 had some effect, consistent with its lack of degrader activity. When compared to the 24-hour results, the effect of ACBI3 appeared unchanged, pointing towards a time-dependent response.
- The procedure was carried out without any problems or deviations from the protocol.

SEPTEMBER 27TH 2026

Overview

- **Date** – Sunday, September 27th, 2026
- **Time (Start–End)** – 8:30- 11:50
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - Taxiarchis Kosmas
- **Project / Subproject** – Cellular effect of pan-KRAS PROTAC in A-549 cells
- **Experiment / Task Title** – Cell Viability assay 72 hours post ACBI3 treatment
- **Objective** – The purpose of this day's experiment was to perform cell viability assays 72 hours post ACBI3 treatment

Experimental documentation

- **Materials / Reagents Used**
  - 50 mL Falcon (x1)
  - 10 µL tips (x21)
  - 200 µL tips (x33)
  - 1 mL tips (x28)
  - 1,5 mL tubes (x21)
  - 10 mL stripettes (x2)
  - Trypan Blue (210 µL)
  - PBS 1X (1,05 mL)
  - FBS (3,1 mL)
  - Trypsin-EDTA (1,05 mL)
  - DMEM, 15% FBS, 1% p/s (20 mL)

Observations & Results

- Microscopic examination of the plates was carried out on the final day of treatment, 72 hours after the compounds were added. Vehicle-treated cells were approximately 60% confluent and retained their typical adherent, epithelial appearance, with some regions becoming crowded due to the longer culture time. By contrast, wells containing ACBI3 displayed visibly fewer cells. The inactive analogue cis-ACBI3 produced a noticeable difference compared to the DMSO wells.
- For harvesting, both the spent medium and the PBS rinse were kept from every well, ensuring that any cells that had already lifted off were counted too. Trypsin-EDTA was used to release the attached cells, and each sample was resuspended by gentle pipetting. Cell suspensions were combined with an equal volume of Trypan Blue, allowing live cells (clear) to be told apart from dead cells (blue) during counting.
- Treatment with the active degrader led to a reduction in viable cell numbers. Taken together with the 24- and 48-hour data, these results show an effect that builds up over time.
- Everything ran smoothly, and the protocol was followed without any deviations.

SEPTEMBER 28TH 2026

Overview

- **Date** – Monday, September 27th, 2026
- **Time (Start–End)** – 8:30- 11:50
- **Name / Initials** – Margarita Papastergiou (MP)
- **Shadowing** - -
- **Project / Subproject** – Cellular effect of pan-KRAS PROTAC in A-549 cells
- **Experiment / Task Title** – Cell Viability assay 96 hours post ACBI3 treatment
- **Objective** – The purpose of this day's experiment was to perform cell viability assays 96 hours post ACBI3 treatment

Experimental documentation

- **Materials / Reagents Used**
  - 50 mL Falcon (x1)
  - 10 µL tips (x21)
  - 200 µL tips (x33)
  - 1 mL tips (x28)
  - 1,5 mL tubes (x21)
  - 10 mL stripettes (x2)
  - Trypan Blue (210 µL)
  - PBS 1X (1,05 mL)
  - FBS (3,1 mL)
  - Trypsin-EDTA (1,05 mL)
  - DMEM, 15% FBS, 1% p/s (20 mL)

Observations & Results

Observations

- On the last harvest day, 96 hours after treatment, the wells were first checked under the microscope. ACBI3-treated wells contained clearly fewer cells, many rounded and floating cells, as well as the cis-ACBI3-treated wells.
- Cells were harvested following the same procedure as at the earlier time points. Medium and PBS washes were saved from each well to retain detached cells, the remaining monolayer was lifted with Trypsin-EDTA, and the cells were resuspended carefully. Samples were then stained with Trypan Blue at a 1:1 ratio and counted, with unstained cells scored as alive and blue cells as dead.
- At this final time point, ACBI3 caused a drop in viable cell numbers relative to the control, and cis-ACBI3 showed effects, confirming that the activity observed needs to be further investigated. Over the entire 24 to 96 hour time course, ACBI3 produced an effect that grew stronger by time and concentration.
- No issues arose during the procedure, and no changes were made to the protocol.

SEPTEMBER 29TH 2026

Overview

- **Date** – Thursday, September 29th, 2026
- **Time (Start–End)** – 13:00 - 15:00
- **Name / Initials** - Margarita Papastergiou / MP
- **Shadowing** - -
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Observation & medium change of A-549 cells
- **Objective** – The purpose of this day's experiment was to observe the A-549 cells in all cell culture flasks and change the medium in the T75-1 and T25 flask.

Experimental documentation

- **Materials / Reagents Used**
  - DMEM, 15% FBS, 1% p/s (14,5 mL)
  - 10 mL stripettes (x2)
  - 5 mL stripettes (x2)
  - 50 mL falcon (x1)

Observations & Results

Observations / Notes

- The confluency observed in each flask was around:
  - T25: 60%
  - T75-1: 60%
  - T75-2: 40%
- The medium color was slightly more orange than the previous day.
- No abnormalities were noted.
- The residual medium in each flask was:
  - T25: 5,8 mL
  - T75-2: 9,5 mL

Results

- The medium was changed in the T25 & T75-1 flasks.

SEPTEMBER 30TH 2026

Overview

- **Date** – Wednesday, September 30th, 2026
- **Time (Start–End)** – 13:00 - 15:00
- **Name / Initials** – Margarita Papastergiou / MP
- **Shadowing** - -
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Observation & medium change of A-549 cells
- **Objective** – The purpose of this day's experiment was to observe the A-549 cells in all cell culture flasks and change the medium in the T75-2.

Experimental documentation

- **Materials / Reagents Used**
  - DMEM, 15% FBS, 1% p/s (9 mL)
  - 10 mL stripettes (x2)
  - 50 mL falcon (x1)

Observations & Results

Observations / Notes

- The confluency observed in each flask was around:
  - T25: 70%
  - T75-1: 65%
  - T75-2: 55%
- The medium color was slightly more orange than the previous day.
- No abnormalities were noted.
- The residual medium in each flask was:
  - T75-2: 9,4 mL

Results

- The medium was changed in the T75-2 flask.

OCTOBER 1ST 2026

Overview

- **Date** – Thursday, October 1st, 2026
- **Time (Start–End)** – 13:50 - 18:15
- **Name / Initials** – Alexandros Vlachos (AV)
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Observation & medium change of A-549 cells
- **Objective** – The purpose of this day's experiment was to observe the A-549 cells in all cell culture flasks and change the medium in the T75-2 and T25 flask.

Experimental documentation

- **Materials / Reagents Used**
  - DMEM, 15% FBS, 1% p/s (14,5 mL)
  - 10 mL stripettes (x2)
  - 5 mL stripettes (x3)
  - 30ml Falcon tubes (x1)

Observations & Results

Observations / Notes

- The confluency observed in each flask was around:
  - T25: 80%
  - T75-1: 30%
  - T75-2: 35%
- The medium color was slightly more orange than the previous day.

Results

- The medium was changed in the T25 & T75-1 flasks.

OCTOBER 2ND 2026

Overview

- **Date** – Friday, October 2nd, 2026
- **Time (Start–End)** – 13:00 - 17:30
- **Name / Initials** – Erriketti Theodora Moumka (EM)
- **Shadowing** - Alexandros Vlachos, Anna Filindri
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Observation, medium change and reculture of A-549 cells
- **Objective** – The purpose of this day's experiment was to observe the A-549 cells in all cell culture flasks, change the medium in the T75-2 flask and split the cellular conents of the T25 flask. Additionally, PenStrep aliquots and fresh complete medium with 15% FBS were prepared (AV).

Experimental documentation

- **Materials / Reagents Used**
  - DMEM, 15% FBS, 1% p/s (15 mL)
  - DMEM, 10% FBS, 1% p/s (2 mL)
  - 10 mL stripettes (x3)
  - 5 mL stripettes (x7)
  - 50 mL Falcons (x2)
  - 30 mL Falcons (x1)
  - 15 mL Falcons (x11)
  - filter (x1)
  - syringe 10 mL (x1)
  - DMEM (42 mL)
  - p/s (0,5 mL)
  - FBS (7,5 mL)
  - 200 uL tips (x10)
  - 1,5 mL tube (x5)
  - Trypan Blue (20 uL)
  - PBS 1X (4 mL)
  - Trypsin-EDTA 0.05% (1 mL)

Observations & Results

Observations / Notes

- The confluency observed in each flask was around:
  - T25: 90%
  - T75-1: 58%
  - T75-2: 50%
- The medium color was slightly more orange than the previous day.
- No abnormalities were noted.

Results

- The cellular contents of the T25 flask were split.
    - ⅓ was recultured in the same flask in 6 mL complete 15% FBS medium
    - ⅔ were made into a pellet stored in the -80 freezer at the molecular lab
- The medium was changed in the T75-2 flask.
- 10 aliquots of 10 mL PenStrep were created from the thawed stock PenStrep bottle. Due to pipetting error, leftover PenStrep volume was stored in three individual 1,5 mL tubes. All aliquots are stored in the -20 freezer, with the tubes stored inside the iGEM box.
- Fresh complete medium with 15% FBS was prepared.

OCTOBER 3RD 2026

Overview

- **Date** – Saturday, October 3rd, 2026
- **Time (Start–End)** – 13:00 - 15:00
- **Name / Initials** – Erriketti Theodora Moumka (EM)
- **Shadowing** - Alexandros Vlachos, Anna Filindri
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Observation & medium change of A-549 cells
- **Objective** – The purpose of this day's experiment was to observe the A-549 cells in all cell culture flasks and change the medium in the T75-1 flask. Additionally, fresh complete media with 15% FBS and with 10% FBS were prepared.

Experimental documentation

- **Materials / Reagents Used**
  - DMEM, 15% FBS, 1% p/s (9 mL)
  - 10 mL stripettes (x5)
  - 5 mL stripettes (x1)
  - 50 mL Falcons (x3)
  - filter (x1)
  - syringe 5 mL (x1)
  - DMEM (88,5 mL)
  - p/s (1 mL)
  - FBS (12,5 mL)
  - 10 uL tips (x4)
  - 200 uL tips (x2)
  - 1000 uL tips (x2)
  - 1,5 mL tube (x2)
  - Trypan Blue (10 uL)

Observations & Results

Observations / Notes

- The confluency observed in each flask was around:
  - T25: 45%
  - T75-1: 55%
  - T75-2: 60%
- The medium color was slightly more orange than the previous day.
- No abnormalities were noted.

Results

- The medium was changed in the T75-1 flask.

- Fresh complete media DMEM 15% FBS 1% P/S, and DMEM 10% FBS 1% P/S were prepared.
- The medium prepared yesterday was assessed for contamination with Trypan Blue staining

OCTOBER 4TH 2026

Overview

- **Date** – Sunday, October 4th, 2026
- **Time (Start–End)** – 13:00 - 15:00
- **Name / Initials** – Erriketti Theodora Moumka (EM)
- **Shadowing** - Alexandros Vlachos, Anna Filindri
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Observation & medium change of A-549 cells
- **Objective** – The purpose of this day's experiment was to observe the A-549 cells in all cell culture flasks and change the medium in the T75-2 and T25 flask.

Experimental documentation

- **Materials / Reagents Used**
  - DMEM, 15% FBS, 1% p/s (14,5 mL)
  - 10 mL stripettes (x2)
  - 5 mL stripettes (x2)
  - 200 uL tips (x4)
  - 1000 uL tips (x1)
  - Trypan Blue (20 uL)

Observations & Results

Observations / Notes

- The confluency observed in each flask was around:
  - T25: 50%
  - T75-1: 65%
  - T75-2: 70%
- The medium color was slightly more orange than the previous day.
- No abnormalities were noted.
- The residual medium in each flask was:
  - T25: 5,9 mL
  - T75-2: 9,6 mL

Results

- The medium was changed in the T25 & T75-2 flasks.
- The media prepared yesterday was assessed for contamination with Trypan Blue staining

OCTOBER 5TH 2026

Overview

- **Date** – Monday, October 5th, 2026
- **Time (Start–End)** – 15:00 - 17:45
- **Name / Initials** – Erriketti Theodora Moumka (EM)
- **Shadowing** - Alexandros Vlachos, Ilias Theocharous
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Observation and reculture of A-549 cells
- **Objective** – The purpose of this day's experiment was to observe the A-549 cells in all cell culture flasks, and split the cellular contents of the T75-1 & T75-2 flasks.

Experimental documentation

- **Materials / Reagents Used**
  - DMEM, 15% FBS, 1% p/s (24 mL)
  - DMEM, 10% FBS, 1% p/s (4 mL)
  - 10 mL stripettes (x4)
  - 5 mL stripettes (x10)
  - 15 mL Falcons (x3)
  - 200 uL tips (x8)
  - 1000 uL tips (x9)
  - 1,5 mL tube (x7)
  - 2 mL tube (x2)
  - Trypan Blue (80 uL)
  - PBS 1X (12 mL)
  - Trypsin-EDTA 0.05% (4 mL)

Observations & Results

Observations / Notes

- The confluency observed in each flask was around:
  - T25: 60%
  - T75-1: 80%
  - T75-2: 85%
- The medium color was slightly more orange than the previous day.
- No abnormalities were noted.
- The residual medium in each flask was:
  - T75-1: 10 mL
  - T75-2: 9,7 mL

Results

- The cellular contents of the T75-1 & T75-2 flasks were split. For each flask:
  - ⅔ were recultured in the same flask in 10 mL complete 15% FBS medium

- ⅓ was pooled with the ⅓ from the other flask and made into two pellets stored in the -80 freezer at the molecular lab

OCTOBER 6TH 2026

Overview

- **Date** – Tuesday, October 6th, 2026
- **Time (Start–End)** – 15:10 - 16:00
- **Name / Initials** – Alexandros Vlachos (AV)
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Observation of A-549 cells and medium change
- **Objective** – The purpose of this day's experiment was to observe the A-549 cells in all cell culture flasks, and change the medium in the T25 flask

Experimental documentation

Materials / Reagents Used

- DMEM, 15% FBS, 1% p/s (6 mL)
- 10 mL stripettes (x2)
- 5 mL stripettes (x2)

Observations / Notes

- The confluency observed in each flask was around:
  - T25: 75%
  - T75-1: 50%
  - T75-2: 60%
- The medium color was slightly more orange than the previous day.
- No abnormalities were noted.

Results

- The medium was changed in the T25 flask.

OCTOBER 7TH 2026

Overview

- **Date** – Wednesday, October 7th, 2026
- **Time (Start–End)** – 15:20 - 17:20
- **Name / Initials** – Erriketti Theodora Moumka (EM)
- **Shadowing** - Ilias Theocharous
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Observation and reculture of A-549 cells
- **Objective** – The purpose of this day's experiment was to observe the A-549 cells in all cell culture flasks, change the medium in the T75-1 & T75-2 flasks, and split the cellular contents of the T25 flask. Additionally, 9 wells were seeded in a 96-well plate to serve as a cell scraping technique trial.

Experimental documentation

- **Materials / Reagents Used**
  - 96-well plate (x1)
  - DMEM, 15% FBS, 1% p/s (24 mL)
  - DMEM, 10% FBS, 1% p/s (3.8 mL)
  - 10 mL stripettes (x5)
  - 5 mL stripettes (x4)
  - 15 mL Falcons (x2)
  - 200 uL tips (x8)
  - 1000 uL tips (x1)
  - 1,5 mL tube (x2)
  - Trypan Blue (20 uL)
  - PBS 1X (2 mL)
  - Trypsin-EDTA 0.05% (1 mL)

Observations & Results

Observations / Notes

- The confluency observed in each flask was around:
  - T25: 85%
  - T75-1: 58%
  - T75-2: 60%
- The medium color was slightly more orange than the previous day.
- No abnormalities were noted.
- The residual medium in the T25 flask was 6.1 mL.

Results

- The medium was changed in the T75-1 and T75-2 cell culture flasks
- The cellular contents of the T25 flasks were split:
  - ⅔ were recultured in the same flask in 6 mL complete 15% FBS medium

- ⅓ was used for seeding of 20.000 cells/well in 9 wells, for a scraping trial in a 96-well plate. A few bubbles were observed after seeding in some wells.

OCTOBER 8TH 2026

Overview

- **Date** – Thursday, October 8th, 2026
- **Time (Start–End)** – 15:10 - 16:35
- **Name / Initials** – Alexandros Vlachos (AV)
- **Shadowing** - Ilias Theocharous
- **Project / Subproject** – Start of A-549 cell line
- **Experiment / Task Title** – Observation of A-549 cells
- **Objective** – The purpose of this day's experiment was to observe the A-549 cells in all cell culture flasks

Experimental documentation

- **Materials / Reagents Used**

  -

Observations & Results

- The confluency observed in each flask was around:
  - T25: 75%
  - T75-1: 85%
  - T75-2: 80%
- The medium color was slightly more orange than the previous day.
- No abnormalities were noted.

OCTOBER 9TH 2026

Overview

- **Date** – Friday, October 9th, 2026
- **Time (Start–End)** – 15:40 - 20:20
- **Name / Initials** – Erriketti Theodora Moumka (EM)
- **Shadowing** - Alexandros Vlachos, Ilias Theocharous
- **Project / Subproject** – Cellular effect of pan-KRAS PROTAC ACBI3 and MDM2 inhibitor Nutlin-3a co-treatment in A-549 cells
- **Experiment / Task Title** – Seeding of A-549 cells
- **Objective** – The purpose of this day's experiment was to seed the A-549 cells in 96-well plates for the upcoming experiment. Additionally, the cellular contents of the T25 flask were made into pellets (AV).

Experimental documentation

- **Materials / Reagents Used**
  - 96-well plates (x2)
  - DMEM, 15% FBS, 1% p/s (21,5 mL)
  - DMEM, 10% FBS, 1% p/s (6 mL)
  - 10 mL stripettes (x4)
  - 5 mL stripettes (x10)
  - 15 mL Falcons (x4)
  - 30 mL Falcons (x1)
  - 200 uL tips (x96)
  - 1000 uL tips (x5)
  - 1,5 mL tube (x6)
  - Trypan Blue (30 uL)
  - PBS 1X (8 mL)
  - Trypsin-EDTA 0.05% (2.5 mL)

Observations & Results

Observations / Notes

- The confluency observed in each flask was around:
  - T25: 75%
  - T75-1: 75%
  - T75-2: 70%
- The medium color was slightly more orange than the previous day.
- No abnormalities were noted.

Results

- The cellular contents of the T75-1 flask were seeded in two 96-well plates at a density of 20k cells/well for the upcoming experiment with assessing the combination effects of N3a and ACBI3 treatment.
    - The seeding was done by dispensing 100-100 µL of the cellular master mix in a zig zag pattern.
    - The outer wells of each plate were filled with sterile dH2O to minimize evaporation during the 96 hrs experimental timeframe.
    - Due to pipetting error, the cellular master mix ran out while the following wells retained half the intended volume: 72H C TR2, 72H N3a TR1, 72H ACBI3 TR2, and 96H C TR1
        - The remaining 10k cells/100 µL medium in these wells were supplemented from the T25 cell culture flask, immediately after.
    - A few bubbles were observed in some of the wells following seeding.
    - All wells were observed under the microscope and reflected similar cell densities, with most cells gathering towards the center rather than the periphery of the well.
- The cellular contents of the T25 flask were split:
    - ⅓ was recultured in 6 mL complete growth medium
    - out of the remaining ⅔, a small volume was used to finish seeding the 96-well plates, while the rest was made into cell pellets stored in the -80 freezer by AV.

OCTOBER 10TH 2026

Overview

- **Date** – Saturday, October 10th, 2026
- **Time (Start–End)** – 15:40 - 20:15
- **Name / Initials** – Erriketti Theodora Moumka (EM), Alexandros Vlachos (AV)
- **Project / Subproject** – Cellular effect of pan-KRAS PROTAC ACBI3 and MDM2 inhibitor Nutlin-3a co-treatment in A-549 cells
- **Experiment / Task Title** – Treatment of A-549 cells with ACBI3, inactive control cis-ACBI3, and Nutlin-3a
- **Objective** – The purpose of today's experiment was to treat A-549 cells with 2,5 μM Nutlin-3a, 50 nM ACBI3, Nutlin-3a + ACBI3, 50 nM inactive control cis-ACBI3, Nutlin-3a + cis-ACBI3.

Experimental documentation

- **Materials / Reagents Used**
  - ❖ DMEM, 10% FBS, 1% p/s (16.148 mL)
  - ❖ 15 mL Falcons (x6)
  - ❖ 10 uL tips (x12)
  - ❖ 200 uL tips (x100)
  - ❖ 1000 uL tips (x10)
  - ❖ 1,5 mL tube (x4)
  - ❖ DMSO (420 uL)
  - ❖ Nutlin-3a 10 mM (stock) (4 μL)
  - ❖ Nutlin-3a 2 mM (stock) (12 μL)
  - ❖ ACBI3 10 mM (stock) (4 μL)
  - ❖ cis-ACBI3 10 mM (stock) (4 μL)
  - ❖ ACBI3 20 μM (stock) (24 μL)
  - ❖ cis-ACBI3 20 μM (stock) (24 μL)

Observations & Results

Observations / Notes

- Both plates were observed following yesterday's seeding.
  - All bubbles observed yesterday had disappeared.
  - All wells were observed: most of the cells had settled, and the pattern of cells gathered more densely near the center than the periphery of the wells was observed once again.

Results

- All the treatment mastermixes for each experimental condition were prepared by EM. Before mastermix preparation, intermediate stock solutions were prepared in DMSO, specifically: 20 μL of N3a 2 mM, 200 μL of ACBI3 20 μM, and 200 μL of cis-ACBI3 20 μM.

- The old medium was removed and the cells in each condition were treated with 200 µL of each corresponding mastermix in a zig zag pattern by AV.
    - Bubbles were observed in multiple wells after treatment.

OCTOBER 11TH 2026

Overview

- **Date** – Sunday, October 11th, 2026
- **Time (Start–End)** – 15:30 - 2:32
- **Name / Initials** – Alexandros Vlachos (AV)
- **Shadowing** - Anna Filindri
- **Project / Subproject** – Cellular effect of pan-KRAS PROTAC ACBI3 and MDM2 inhibitor Nutlin-3a co-treatment in A-549 cells
- **Experiment / Task Title** – Cell viability assay of treated A-549 cells, 24 h post-treatment.
- **Objective** – The purpose of today's experiment was to check the cell viability of treated A-549 cells at different concentrations, 24 hours post-treatment.

Experimental documentation

- **Materials / Reagents Used**
  - **200 µL tips (x9)**
  - **1 mL tips (x2)**
  - **1,5 mL tubes (x18)**
  - **Trypan Blue (180 µL)**
  - **PBS 1X (3mL)**

Results

1. During observation, all the cells in each well seemed healthy.
2. Specifically, about the experiment, some problems arised, many of which required on-site troubleshooting. The combination of them could alter experimental results.

Observations / Notes

1. Initial Observations
   - ➢ Since the flasks are no longer available/necessary the observation procedure was only performed for the first 96-well plate (24hrs & 48hrs)
   - ➢ The cells seemed healthy, though slightly concentrated at the center of each well.
   - ➢ Among all wells, there was no significant difference in confluency
2. During cell collection
   - ➢ For the 24h time point, cells were accidentally collected from top-to-bottom (per condition), while for the other time points the correct procedure was implemented (per TR)
   - ➢ During collection of the cells from the first well (Control TR1) the protocol discussed previously was performed to the letter, but several problems occurred.

- ➢ However, the washing step dislodged some of the cells (noticed after observation). Therefore, the PBS was collected in every well afterwards
- ➢ Due to a substantial amount of bubbling during the wash and presence of red color in C_TR1, the volume of PBS used in for rest of the wells was modified to 150ul
- ➢ Regarding the scraping process, a bent 200ul pipette tip was used to manually detach the cells into the supernatant (PBS). After the first well, the recommended volume of PBS was deemed insufficient for removing the cells easily and safely. After C_TR1 the volume used for scraping was also 150ul
- ➢ Every well (in every time point) was inevitably scraped for different durations, possibly altering the final results

3. Cell Counting
   - ➢ After collection and concentration of each suspension to half its volume, the cells were successfully in a Neubauer chamber (10ul Trypan Blue, 10ul suspension)
   - ➢ The Nutlin3a + ACBI3 TR2 sample was counted twice, because of clumping and/or almost complete absence of cells.
   - ➢ Start time: 23:35

OCTOBER 12TH 2026

Overview

- **Date** – Monday, October 12th, 2026
- **Time (Start–End)** – 17:50 - 0:44
- **Name / Initials** – Alexandros Vlachos (AV)
- **Shadowing** - Ilias Theocharous
- **Project / Subproject** – Cellular effect of pan-KRAS PROTAC ACBI3 and MDM2 inhibitor Nutlin-3a co-treatment in A-549 cells
- **Experiment / Task Title** – Cell viability assay of treated A-549 cells, 48 h post-treatment.
- **Objective** – The purpose of today's experiment was to check the cell viability of treated A-549 cells at different concentrations, 48 hours post-treatment.

Experimental documentation

- **Materials / Reagents Used**
  - **200 µL tips (x93)**
  - **1,5 mL tubes (x18)**
  - **Trypan Blue (210 µL)**
  - **PBS 1X (4.25mL)**

Results

1. In wells treated with Nutlin3a (alone or in combination with another agent) there was a slight -but observable- drop in confluency

2. The experiment went as planned, with fewer alterations than the 24h collection protocol

Observations / Notes

1. Initial Observations
   Ø The wells treated with Nutlin3a, Nutlin3a+ACBI3 and Nutlin3a+cis looked noticeably less confluent compared to other wells

2. During cell collection:

   Ø In contrast to the 24h collection, the protocol was more stable in the 48h time point.
   Ø During the washing step the PBS was always collected (excluding the Nutlin3a TR1 and TR2 wells)
   Ø The wells were scraped and washed more thoroughly in order to avoid repeating the procedure and stressing the leftover cells further
   Ø The concentration of the samples was performed by Ilias Theocharous (volume measurements, centrifugation and gentle removal of supernatant)

3. Cell Counting
   Ø The control samples were counted twice, because of clumping and/or absence of cells.
   Ø Start time: 21:45

OCTOBER 13TH 2026

Overview

- **Date** – Tuesday, October 13th, 2026
- **Time (Start–End)** – 16:45 - 00:00
- **Name / Initials** – Alexandros Vlachos (AV)
- **Shadowing** - Ilias Theocharous
- **Project / Subproject** – Cellular effect of pan-KRAS PROTAC ACBI3 and MDM2 inhibitor Nutlin-3a co-treatment in A-549 cells
- **Experiment / Task Title** – Cell viability assay of treated A-549 cells, 72h post-treatment.
- **Objective** – The purpose of today's experiment was to check the cell viability of treated A-549 cells at different concentrations, 72 hours post-treatment.

Experimental documentation

- **Materials / Reagents Used**
  - **200 µL tips (x92)**
  - **1,5 mL tubes (x18)**
  - **Trypan Blue (180 µL)**
  - **PBS 1X (5.4mL)**

Results

1. Well confluency did not change drastically or in an unexpected manner
2. Cell collection went smoothly and with a finalized protocol

Observations / Notes

1. Observation
   Ø The 72h section of the second plate was observed and photographs were taken.
   Ø Macroscopically, the medium color had changed noticeably
2. Experiment
   Ø The protocol was finalized. The PBS added was the same for all wells. The wash was performed with 150ul PBS and collected, while the scraping was implemented in another 150ul PBS.
   Ø There was an effort to keep the time and force of scraping the same for all wells
   Ø Everything was finished successfully without complications.
   Ø The volume concentration and Neubauer loading was performed by Ilias Theoharous
3. Cell counting
   Ø This time there was no shortage of cells on the cytometer
   Ø A significant amount of clumping was observed
   Ø Start time: 20:30

OCTOBER 14TH 2026

Overview

- **Date** – Wednesday, October 14th, 2026
- **Time (Start–End)** – 17:15 - 2:45
- **Name / Initials** – Alexandros Vlachos (AV)
- **Shadowing** - Anna Filindri
- **Project / Subproject** – Cellular effect of pan-KRAS PROTAC ACBI3 and MDM2 inhibitor Nutlin-3a co-treatment in A-549 cells
- **Experiment / Task Title** – Cell viability assay of treated A-549 cells, 96 h post-treatment.
- **Objective** – The purpose of today's experiment was to check the cell viability of treated A-549 cells at different concentrations, 96 hours post-treatment.

Experimental documentation

- **Materials / Reagents Used**
  - **200 µL tips (x93)**
  - **1,5 mL tubes (x18)**
  - **Trypan Blue (210 µL)**
  - **PBS 1X (5.4mL)**

Results

1. Several differences were observed in the microscope before cell collection
2. The entirety of the experiment went smoothly

Observations / Notes

1. Observation
   Ø The wells designed for the 96h time point were observed
      i. The wells treated with only Nutlin3a had a very obvious drop in confluency
      ii. The wells treated with ACBI3 also had a slight drop in confluency
      iii. The wells with the largest drop were the ones treated with Nutlin3a combined with ACBI3
   Ø Macroscopically, the medium seemed spent and the volume was noticeably reduced
2. Experiment
   Ø No changes were applied to the established protocol (150ul wash + 150ul scraping)
   Ø The Control TR1 well was scraped for a longer, which might have possibly altered the viability and growth counts
   Ø Every one of the remaining wells was scraped thoroughly and for a similar time period
   Ø The volume concentration was performed mostly by Anna Fillindri
3. Cell Counting

Ø Difficulties caused by clumping were virtually non-existent in the 96h time point
Ø The Control TR1 and TR2 samples were counted twice, since the cell number was very low on the first count (probably due to insufficient mixing before loading)
Ø Start time: 22:45