Adherent cell culture

Technic: HEK293T adherent cell culture

Goal: To maintain the cell line alive and in good condition

Procedure:

This example is based on a culture dish of **100 cm²** and a **culture volume of 10 mL**, but it can be applied to other culture volumes (Dish 35 / 60 / 100 / 150 mm or T25, T75, T150 in our case).

1. Prepare DMEM medium with 10% Fetal Bovine Serum (FBS)
   (+ 1% Penicillin-Streptomycin if needed).
   For example, for **50 mL** of medium, mix **45 mL DMEM**, **5 mL FBS**, and **500 µL of 100X Penicillin-Streptomycin**.
2. Prewarm the complete medium in a 37°C water bath.
3. Remove the current culture medium and discard it.
4. Wash with **1–2 mL of sterile PBS**.
   ⚠️ Be careful! The cells detach easily. Do not squirt directly on the cells; let the PBS flow along the edge of the flask.
5. If necessary: add **0.5 mL of 1X Trypsin-EDTA** by gentle pipetting on the cells to detach them (detachment is visible to the naked eye or under an inverted microscope).
   Immediately inactivate trypsin with **9.5 mL of complete medium**.

   Otherwise: detach the cells using **1 mL of fresh complete medium**, then add it to **9 mL of the same medium**.

6. Pipette up and down several times to separate the cells and homogenize the solution.
7. If necessary: take **50 µL** of the culture to count the cells in order to determine the cell concentration.
8. Inoculate the cells at the desired concentration into new culture dishes.
   For example, starting from a **100 mm cell culture dish** at **100% confluence** (= 3.10^6 cells/mL), we set it at **20%** by diluting the cell suspension 1:5.
   For 10 mL, this corresponds to **2 mL of the cell solution + 8 mL of fresh medium**.
9. Incubate at **37°C**, **5% CO_2**.

Reagents and Solutions:

- DMEM + 10% FBS + 1% Penicillin-Streptomycin (100X)
- If needed: Trypan Blue

- Sterile PBS
- Trypsin (+EDTA, though not strictly necessary)

Equipment:
- Water bath
- Laboratory aspiration system (e.g. VacuSafe)
- Microscope
- 15 mL or 50 mL sterile tubes
- Serological pipettes
- Pipetboy
- If needed: cell counting system like QuickRead slide or Malassez counting chamber
- Culture flask or dish

Estimate time: ~30/45 minutes

Controls and Troubleshooting:
Check your cells every day to monitor their growth and to prevent any problem before it's too late for the cells.

When the medium turns yellowish, check:
- Cell confluence: they might be too crowded,
- Possible contamination. This can be seen with the naked eye or under the microscope. Perform additional tests if nothing is visible after a few days.

Avoid going below 10% confluence when inoculating, as the cells might not grow properly.

After having already measured the cell concentration at 100% confluence s**everal times, and if this value is stable,** it is not necessary to recalculate the number of cells each time: you can rely on your previously obtained values.

Remember to turn on the water bath as soon as you enter the culture room — it will save you from waiting for it to reach temperature!

Suspension cell culture

Technic: HEK293F suspension cell culture

Goal: To maintain the cell line alive and in good condition

Procedure:

This example is based on a culture dish of **100 cm²** and a **culture volume of 10 mL**, but it can be applied to other culture volumes (Dish 35 / 60 / 100 / 150 mm or T25, T75, T150 in our case).

1. Warm the Freestyle 293 medium in a water bath to 37°C.
2. Take 50 µL of the culture to count the cells in order to determine the concentration. Transfer the sample to an Eppendorf tube.

   ⚠️ Use a serological pipette to collect the cells to avoid contamination with the micropipette.

3. Add 50 µL of Trypan blue to the tube. Mix by pipetting 2-3 times.
4. Place 50 µL of the mixture on the slide and count immediately (be careful, it dries quickly, especially in summer).
5. The cells should have a regular morphology: spherical cells, isolated or in small aggregates. The maximum recommended concentration is 2.10^6 cells/mL. At this point, the cells must be split.
6. Dilute the cells in fresh medium to return to a range of 0.25 to 0.5 x 10^6 cells/mL in a new culture flask.
7. If necessary, dispose of excess cell culture in a liquid waste container with bleach.
8. Incubate with agitation at 37°C, 5% CO_2, at 135 rpm.

Reagents and Solutions:

- Freestyle 293 medium
- Trypan Blue

Equipment:

- Water bath
- Laboratory aspiration system (e.g. VacuSafe)
- Microscope
- Eppendorf tubes
- Serological pipettes
- Pipetboy
- cell counting system like QuickRead slide or Malassez counting chamber

- Shaking flask (eg. 125 for 25mL of culture)

Estimate time:
~30/45 minutes

Controls and Troubleshooting:

Check your cells every day to monitor their growth and to prevent any problem before it's too late for the cells. This is really important as the medium isn't colored so you won't see any changes with the naked eye.

Contamination can occur. If the medium becomes very cloudy, that's when you need to worry.

Remember to turn on the water bath as soon as you enter the culture room — it will save you from waiting for it to reach temperature!

Be careful because the medium can evaporate, so remember to check the volume of the culture regularly (every 2-3 days).

We did not have an effective shaking system, so we did not continue the experiments with this cell line.