Culture of cells pancreatic

Technic: Culture of pancreatic cell lines MIA PaCa2, BxPC3, and Panc-1

Goal: To maintain the pancreatic cell lines alive and in good condition

Procedure:
For the passage of a 100mm cell culture dish at 10% confluence:

1. Preheat the RPMI medium supplemented with 10% FBS and 1% Penicillin/Streptomycin (100X) in a 37°C water bath.
2. Remove the current culture medium.
3. Wash the cells with 1-2 mL of sterile 1X PBS. Do not pipette directly onto the cells, let the solution run along the edge of the Petri dish.
4. Add 1 mL of Trypsin-EDTA and incubate for 1–2 minutes at room temperature. Monitor cell detachment under the microscope.
5. Once detachment is observed, immediately inactivate trypsin by adding 2 mL of complete medium and gently pipette up and down to fully dissociate the cells.
6. Transfer the 2 mL of cell suspension into a sterile tube containing 8 mL of complete medium.
7. Take 50 µL of this suspension for cell counting.
8. Prepare a new dilution: collect 1.5 mL and add to 8.5 mL of complete medium, then resuspend.
9. Transfer the 10 mL of cell suspension into a new 100mm cell culture dish gently.
10. Place the dish in a 37°C, 5% CO_2 incubator.
11. Perform cell counting using a QuickRead slide or Malassez chamber with Trypan Blue staining.

Reagents and Solutions:

- Complete medium: RPMI + 10% FBS + 1% Penicillin/Streptomycin (100X)
- Sterile 1X PBS
- Trypsin-EDTA solution
- Trypan Blue

Equipment:

- Water bath
- Laboratory aspiration system (e.g. VacuSafe)
- Microscope
- 15 mL sterile tubes
- Serological pipettes
- Pipetboy

- QuickRead slide or Malassez counting chamber
- 100mm cell culture dish

Estimate time: ~30/45 minutes

Controls and Troubleshooting:
Let the trypsin act at 37°C for 5 minutes, checking cell detachment every minute under the microscope.
Gently tap the dish against the edge of the bench to create a shear force parallel to the surface and help detach the cells.
Avoid going below 15% confluence when inoculating, because they might not grow properly.

100 mm cell culture dish = 10 mL
60 mm cell culture dish = 5 mL
35 mm cell culture dish = 2 mL
Millicell = 1-2 mL
96 wells = 200-300 µL
6 wells = BP35

Cell culture dish	Surface	MiaPaCa2	PanC1	BxpC3	confluence
60 mm	28 cm^2	5,8.10^5 cells/mL 2,9.10^6 cells	4,1.10^5 cells/mL 2,05.10^6 cells	4,5.10^5 cells/mL 2,25.10^6 cells	100%
100 mm	75 cm^2	8,7.10^6 cells	6,15.10^6 cells	6,75.10^6 cells	100%
35 mm	9.5 cm²	9,8.10^5 cells	6,9.10^5 cells	7,7.10^5 cells	100%
96 wells	0,36 cm^2	4,1.10^4 cells	2,9.10^4 cells	3,2.10^4 cells	100%