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Adherent Cell Culture (generic)

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Miller Group - cell culture

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Protocol status: Working
We use this protocol and it's working

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Abstract

Standard procedure for culturing adherent cell lines. Follow these instructions and cell type-specific instructions on the **Cell line information** protocol

Guidelines

Maintain sterile culture technique throughout.

Avoid reaching pipettes inside stock bottles or flasks - use a pipette gun and stripette to minimize the risk of contamination.
Avoid touching the neck of the flask with your pipette - this is a primary source of contamination.
Avoid liquid touching the filter inside the flask and pipette gun. If they are touched, replace the pipette gun filter and flask lid.

When new to this technique, check your proficiency by growing cells without antibiotic. Infections will rapidly arise if there is a problem. Deep clean incubators, biological safety cabinets and pipette guns (swapping out their filters) if an infection arises to avoid it spreading.

Maintain a cell culture log with calculated doubling times to identify if there are changes to the growth pattern of your cells. Changes to growth patterns can indicate infections or inappropriate split ratios (cells reaching confluence too quickly or being seeded too sparsely).

Materials

Trypsin (0.05%) e.g. Gibco Cat. No. 25300054.
Phosphate buffered saline (PBS) e.g. Sigma Aldrich Cat. No. D8537-500ML
Complete cell culture media (see: **dx.doi.org/10.17504/protocols.io.rm7vz82o8vx1/v2**)
Tissue-culture treated cell culture flasks (T25, T75, T150) e.g. Thermo Fisher Scientific Cat. No. 156499
Motorized pipette controllers, also known as a "pipette gun" e.g. Eppendorf Easypet Cat. No. 4430000018
Serological pipettes e.g. Corning stripettes Cat. No. 4488

Before start

Warm complete media in 37^{O}C water bath for at least 20 minutes to avoid shocking the cells.
Sterilize the hood, ensure proper airflow.
Wear a labcoat and gloves.

1 Remove spent media from flask.

2 Add 5-10 mL of PBS. Gently swirl flask to rinse cells then remove PBS.
- this removes serum proteins that might interfere with trypsin

3 Add trypsin (0.05%, Gibco Cat. No. 25300054)
- 1 mL for T25
- 2.5 mL for T75
- 5 mL for T150

4 Incubate flask at 37^{o}C for 5-10 min.
- check the flask at 5 min by holding it up to the light, if cells fall off by themselves, culture is ready, if some remain stuck, continue incubating up to 10 min

5 Add 4x volume of complete media into flask. Pipette gently up and down to collect dislodged cells. Transfer cells to a 15 mL conical tube.
- 4 mL for T25
- 10 mL for T75
- 20 mL for T150

6 Centrifuge cells for 4 minutes at 300g.

7 Add appropriate volumes of complete media into a new flask. Label date, passage number and initials on flask.
- 7 mL for T25
- 13 mL for T75
- 30 mL for T150

8 After centrifuging, remove supernatant. Break up cell pellet by tapping the tube. Resuspend cells with 1-2 mL of complete media. Transfer appropriate fraction of cell suspension into flask for the appropriate split ratio as indicated by 'Cell line information' protocol (**dx.doi.org/10.17504/protocols.io.rm7vz82o8vx1/v2**)

Protocol references

Thermo Fisher Scientific has youtube video resources demonstrating these procedures :
https://www.youtube.com/playlist?list=PLGlvFEwL2wDGAFJFFFyi-LL1zu64BHvxv