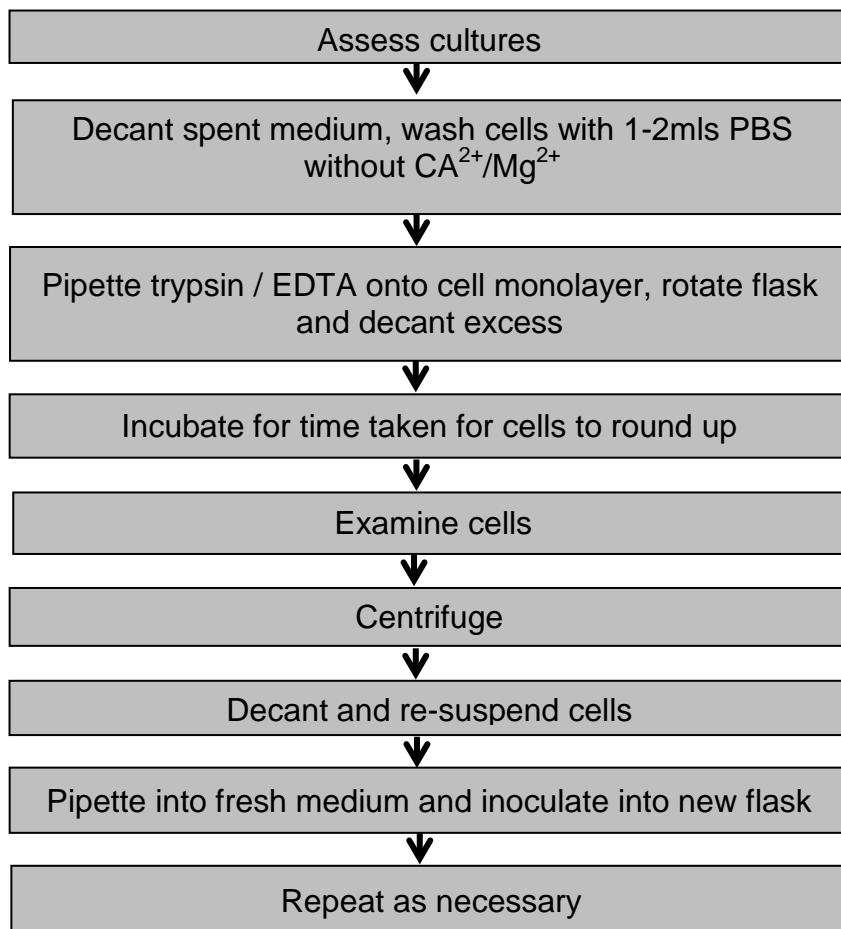


Cell culture protocol:

Subculture of semi-adherent cell lines



Aim

Some cultures grow as a mixed population (e.g. B95-8 - marmoset) where a proportion of cells do not attach to the tissue culture flask and remain in suspension. Therefore to maintain this heterogeneity both the attached cells and the cells in suspension must be sub-cultured.

Materials

- media pre-warmed to 37°C (refer to the ECACC Cell Line Data Sheet for the correct medium)
- 70% (v/v) isopropanol in sterile water
- PBS without Ca²⁺/Mg²⁺
- 0.25% trypsin/EDTA in HBSS, without Ca²⁺/Mg²⁺
- Soyabean Trypsin Inhibitor
- Trypan blue (vital stain)

Equipment

- personal protective equipment (sterile gloves, laboratory coat, safety visor)
- Waterbath set to appropriate temperature
- Microbiological safety cabinet at the appropriate containment level
- Centrifuge

Cell culture protocol:

Subculture of semi-adherent cell lines

- Inverted phase contrast microscope
- Incubator
- Haemocytometer
- Pre-labelled flasks
- Marker Pen
- Pipettes

Procedure

1. View cultures using an inverted phase contrast microscope to assess the degree of confluency and confirm the absence of bacterial and fungal contaminants. Give the flask a gentle knock first, this may dislodge the cells from the flask and remove the need for a trypsinisation step loss of some cells due to associated washes
2. Decant medium containing non-adherent cells into a sterile centrifuge tube and retain
3. Wash any remaining attached cells with PBS without $\text{Ca}^{2+}/\text{Mg}^{2+}$ using 1-2ml for each 25cm^2 of surface area. Retain the washings
4. Pipette trypsin/EDTA onto the washed cell monolayer using 1ml per 25cm^2 of surface area. Rotate flask to cover the monolayer with trypsin. Decant the excess trypsin
5. Return flask to incubator and leave for 2-10 minutes
6. Examine the cells using an inverted microscope to ensure that all the cells are detached and floating. The side of the flasks may be gently tapped to release any remaining attached cells (see Protocol 6 - Cell Quantification)
7. Transfer the cells into the centrifuge tube containing the retained spent medium and cells
8. Centrifuge the entire cell suspension at $150 \times g$ for 5 minutes
9. Remove the supernatant and re-suspend the cell pellet in a small volume (10-20ml) of fresh culture medium. Count the cells
10. Pipette the required number of cells into a new labelled flask and dilute to the required volume using fresh medium (refer to ECACC Cell Line Data Sheet for the required seeding density)
11. Repeat this process every 2-3 days as necessary

Key Points

1. Although most cells will detach in the presence of trypsin alone the inclusion of EDTA enhances the activity of the enzyme by removing inhibitory cations
2. Trypsin is inactivated in the presence of serum. Therefore, it is essential to remove all traces of serum from the culture medium by washing the monolayer of cells with PBS without $\text{Ca}^{2+}/\text{Mg}^{2+}$. Repeated warming to 37°C also inactivates trypsin
3. Cells should only be exposed to trypsin/EDTA long enough to detach cells. Prolonged exposure could damage cell surface receptors. In general, a shorter time of exposure to trypsin is required for semi-adherent cell lines in comparison to adherent cell lines
4. Trypsin should be neutralised with serum prior to seeding cells into new flasks otherwise cells will not attach
5. Trypsin may also be neutralised by the addition of Soyabean Trypsin Inhibitor, where an equal volume of inhibitor at a concentration of 1mg/ml is added to the trypsinised cells. The cells are then centrifuged, re-suspended in fresh culture medium and counted as above
6. If a CO_2 incubator is not available gas the flasks for 1-2 minutes with 5% CO_2 in 95% air filtered through a $0.2\mu\text{m}$ filter.