Mack Cell Cryopreservation

General Notes

- DMSO is toxic to cells at room temperature, freeze cells soon after they are resuspended in freezing media

Materials

- Mack cells, in culture
- Mack growth media (Mack GM)
- 0.05 or 0.25% trypsin-EDTA solution
- DPBS, no Ca, no Mg
- Cyrovials, labeled
- Mr. Frosty™ Freezing Container
- Liquid nitrogen (LN_2)

Method

1. Warm growth media to room temperature.
2. View cells under light microscope and note observations.
3. Thaw trypsin from the -20°C to room temperature.
    a. The volume will depend on the surface area of plates/flasks from which you are passaging. Recommended volumes are shown the reference volumes table at the bottom of this protocol.
4. Once the media and trypsin are warm, carefully aspirate the spent media (i.e., media that remains after the cells have utilized the components) from the flask using an aspirating pipette connected to the vacuum inside the biosafety cabinet.
    a. NOTE: For greater precision, a 200 µL micropipette tip can be placed on the tip of the aspiration pipette.
5. Add the appropriate volume of sterile DPBS (see reference volumes table) using a serological pipette.
    a. This step washes off residual growth media, as FBS can inhibit trypsin.
6. Carefully aspirate DPBS using an aspirating pipetted connected to vacuum in the biosafety cabinet.
7. Add the appropriate volume of warmed trypsin (see reference volumes table) using a serological pipette. Tilt the culture vessel gently to ensure even distribution.
8. Incubate for 5 minutes at room temperature
9. After 5 minutes, check cell attachment on the culture vessel using the microscope.
    a. The cells should appear circular and detached (Fig. 1). If cells are circular but not detached, firmly tap (~5 times) each side of flask to help cells detach. Wait (~30 sec) and repeat as necessary until no/very few cells are visibly attached under microscope.
    b. NOTE: Once detached, cells are in suspension and frequently will be moving quickly across field of view. To test if cells detached, gently agitate flask and observe if cells remain in place (i.e., do not move with flow of liquid movement).

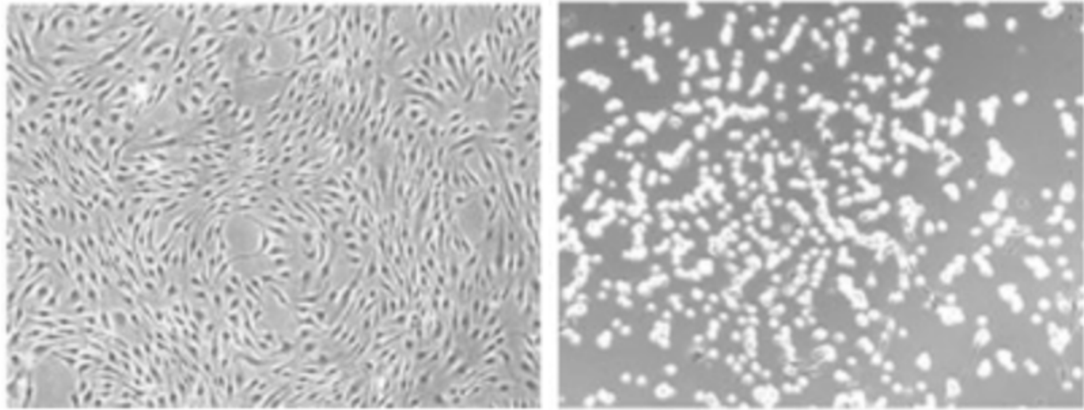

Fig. 1: Example images of adherent cell culture before (left) and after (right) cell detachment with trypsin[1]

10. Immediately after observing no/very few visibly attached cells, bring flask back to biosafety cabinet and add an equal volume (in comparison to the volume of trypsin) of growth media using a pipette. Using the same pipette, rinse the flask surface by pipetting up and down (~5 times), dispensing directly onto entire bottom of flask to help cells detach. Transfer cell suspension to labeled (species/cell type, passage number, initials, date) tube.
    a. NOTE: Be quick about adding GM as soon as you observe no/very few visibly attached cells; the longer the trypsin is left with the cells, the more cells can be damaged by excessive exposure to protease. Adding media (which includes FBS) neutralizes trypsin and inactivates its proteolytic activity.
    b. NOTE: Since adding the growth media neutralizes the trypsin, it is also important to move the cells out of the culture vessel, as they could re-adhere to the tissue culture treated plastic (but will not adhere to the conical tube's plastic).
11. Spin the sterile cell suspension in the centrifuge at 300 RCF for 5 minutes.
12. Bring the centrifuged cells into the biosafety cabinet.
13. Carefully aspirate off supernatant without disturbing cell pellet using aspirating pipette connected to vacuum in biosafety cabinet.
14. Resuspend the cell pellet in 2-10 mL of growth media. Mix well by pipetting up and down.
    a. Note: The volume of media will depend on the expected cell number. For cryopreservation, we typically aim for 2-5 million cells/cm^2
15. Count the cells on your cell counter.
16. Dilute cell suspension with Mack GM to ~1.11 million cells/mL.
    a. Cells will be cryopreserved at 1 million cells/mL. The number of million cells will be the needed number of cryovials, each with 1 million cells in 1 mL.
    b. The freezing medium contains 90% Mack GM and 10% DMSO.
17. Add DMSO such that the final cell concentration is 1 million cells/mL.
    a. DMSO should account for 10% of the final solution volume.
18. Add 1 mL of cell suspension to each cryovial.
19. Move to freezing container and place in -80ºC.
20. The next day, move cells to LN_2 for indefinite storage.
21. Generally, we recommend seeding 3,000-8,000 cells/cm^2.