

Subculture of Monolayer Culture

Outline

Remove the medium and wash cells with D-PBS. Briefly expose cells to trypsin and incubate for 10 minutes. Disperse cells in fresh growth medium and reseed the cells.

Materials

Sterile

- Cell culture
- Culture flasks, T25, T75, or T150
- Growth medium
- D-PBS without calcium and magnesium (Cat. No. 21-031-CV, Mediatech)
- Trypsin, 0.25% with 2.21 mM EDTA in HBSS (Cat. No. 25-053-CI, Mediatech)
- Pipettes, 2 mL, 5 mL, 25 mL

Nonsterile

- Gloves
- Pipette Aid
- Alcohol, 70% in spray bottle
- Materials for cell counting (*see protocol for cell counting -- optional*)

Protocol

1. Prepare hood by swabbing with 70% alcohol
2. Collect materials and reagents and place them in biosafety hood, swab with 70% alcohol
3. Defrost Trypsin to 4°C in fridge, and prewarm medium and D-PBS in water bath to 37°C
4. Retrieve culture flask from the incubator
5. Examine cells on an inverted microscope: look for signs of cell deterioration or contamination
6. Check criteria for subculture: ~70% confluency and otherwise healthy looking cells
7. Place culture flask in biosafety hood
8. Remove the medium by aspirating
9. Add 0.2 mL/cm² of D-PBS to the side of the flask opposite the cells
10. Rinse the prewash over the cells and discard – this removes any serum that would inhibit trypsin
11. Add 0.1 mL/cm² of trypsin and ensure that monolayer is completely covered
12. Leave stationary for 15-30 sec (don't try to loosen cells by tapping the flask)
13. Withdraw all but a few drops of trypsin by aspirating
14. Incubate at 37°C for 10 min, or until cells round up and monolayer slides when flask is tilted
15. Add 0.1 mL/cm² of medium and disperse cells by repeated pipetting over the monolayer
16. Pipette cell suspension up-and-down a few times to create single-cell suspension (don't create foam)
17. Withdraw about 100 µL of cell suspension into microfuge tube for cell counting (OPTIONAL)
18. For fixed split ratio: add appropriate amount of medium and reseed at 0.2 mL/cm²
19. Replace caps on culture flasks, and gently rock the flasks to distribute cells evenly
20. Label the flasks, initial and date
21. Place flasks in the incubator
22. Clean hood by swabbing with 70% alcohol