

Subculturing Cells

Materials

1. Medium with supplements (serum, glutamine, antibiotics).

2. STE, 37°C. Stored at 4°C.

| | 4000 ml | 1000 ml |
|---------------------------|---------|---------|
| NaCl 150 mM | 35.1 g | 8.8 g |
| Tris HCl 44 mM | 28.1 g | 7.0 g |
| Tris Base 6 mM | 2.7 g | 0.68 g |
| Na ₂ EDTA 1 mM | 1.5 g | 0.38 g |

Adjust pH to 7.2.

Sterilize by autoclaving.

Store at 4°C.

3. Trypsin, 0.25% or 0.05%, 37°C. Stored at -20°C. Do not leave trypsin in waterbath for an extended period of time.

4. Pipets: 10 ml, 5 ml (optional), Pasteur pipets. Pipeting aid.

5. Miscellaneous items: pipeting aids, tray for used glassware, Sharpie. See general guidelines for cell culture.

6. Fresh culture dishes.

Procedure

1. Set up necessary items in the hood. Spray the exterior surface of containers with 70% alcohol. Also sterilize your hands.

2. Remove all medium from the stock dish.

3. Add STE, ~5 ml for 100 mm dish, ~3 ml for 60 mm dish. Rinse the entire surface by rocking the dish.

4. Remove STE.

5. Add ~1 ml trypsin with a Pasteur pipet. Rock the dish gently and remove all the trypsin right away (using the same pipet).

6. Set up new dishes and add an appropriate amount of medium, ~10 ml for 100 mm dishes, ~4 ml for 60 mm dishes, ~2-2.5 ml for microinjection dishes.

7. Check cells under the scope. Add 2-5 ml medium when almost all cells are round. Do not leave cells in trypsin much longer than necessary.

8. Gently blow cells off the surface of the dish. Rotate the dish to recover cells from the entire surface.

9. Add an appropriate volume of the cell suspension to the fresh dishes. Gently rock/swirl the dish to spread out the cells.

10. Clean up the hood and the vacuum suction line.
