Materials and Equipment

Materials

- Fibroblasts (AU009-F, AI001-F or AE009-F)
- Fibroblast Cellutions Medium (I-GRO)
- Fetal Bovine Serum (FBS)
- 1× PBS (Ca^{2+}/Mg^{2+}-free)
- TrypLE Express or equivalent enzyme solution
- 15 ml and 50 ml conical centrifuge tubes
- Tissue culture vessels

Equipment

- Biosafety Cabinet
- Water Bath, 37°C
- Centrifuge
- Incubator, 37°C, 5% CO_2
- Pipet-Aid and Pipettes

Procedure

1.	Immediately upon delivery • Remove vial from shipping container and check that it is still frozen—if thawed, contact DV Biologics. • Transfer frozen vial immediately to liquid nitrogen until you are ready to thaw and begin cell culture.

2.	Thawing Cells
	• Perform and maintain all cell culture using aseptic techniques.
	• Prepare I-GRO medium. After mixing, store complete medium at 4°C for a maximum of 30 days.
	• Aliquot and warm only media required that day at 37°C.
	• Thaw cells rapidly and with agitation (See How to Thaw Cells).
	  ○ Slowly dilute thawed cells with 3- to 10-fold excess cold FBS (or complete I-GRO medium).
	  ○ Centrifuge at 400 g for 5 minutes.
	• Remove supernatant and disperse cell pellet in fresh 37°C warm I-GRO.
	• Count cells (see How to Count Fresh Cells).

3.	Plating and Maintaining Cells • Prepare an aliquot of cells to plate at a density of 3.0 x 10^3cells/cm^2. • Carefully add cell suspension to cell culture vessel with no coating. • Place cell culture flasks/plates in incubator at 37°C and 5% CO_2. • Feed cells every 2-3 days by completely replacing the medium. Observe cells daily. • Once cells become 60-70% confluent, feed more regularly (every other day). • When cells are nearing confluency (> 80%), prepare vessels for passage/expansion. NOTE: Do not let cells become overly confluent.
4.	Passaging Cells (for Details see How to Passage Cells). • Aspirate and discard medium; wash cells with PBS without Ca^{2+} and Mg^{2+}, and aspirate. • Add TrypLE Express or equivalent enzyme solution at a volume that just covers the cell monolayer. • Incubate at 37°C; microscopically observe detachment of cells every 5 min; avoid toxic effects of enzymatic over-digestion. • Quench the enzyme digestion by adding 2- to 5-fold volume of I-GRO medium to the detached cells in enzyme solution. • Transfer the entire resulting cell suspension from the culture vessel to a centrifuge tube. • Centrifuge the cell suspension at 400 g at 4°C for 5 min. Aspirate supernatant. • Disperse the cell pellet in fresh 37° warm I-GRO, and take sample of this suspension to count cells. • To propagate cells, seed cells in culture vessels at a density of 3.0 x 10^3 cells/cm^2 in I-GRO medium. • If more cells are obtained than needed for re-plating, the additional cells may be frozen. (See How to Freeze Cells).