Materials and Equipment

Materials

- Chondrocytes (AM006-F)
- Fibroblasts Cellutions Media (I-GRO)
- TGF-β-3 (10 ng/ml, final concentration)
- Fetal Bovine Serum (FBS)
- 1× PBS (Ca^{2+}/Mg^{2+}-free)
- TrypLE Express or equivalent enzyme solution
- 15.0 ml and 50.0 ml conical centrifuge tubes
- Tissue culture vessels
- Dimethyl Sulfoxide (DMSO), if freezing cells

Equipment

- Biosafety Cabinet
- Water Bath, 37°C
- Centrifuge
- Incubator, 37°C, 5% CO^2
- Pipet-Aid and Pipette

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 for use that day at 37°C. Add fresh TGF-β-3 (10 ng/ml, final concentration) to I-GRO aliquot to be used that day for chondrocytes.
	• 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 medium with TGF-β-3.
	• Count Cells (see How to Count Fresh Cells).

3.	Plating and Maintaining Cells • Prepare an aliquot of cells to plate at a density of 3.0-5.0 x 10^3cells/cm^2. • Carefully add cell suspension to cell culture vessel with no coating. • Place cell culture vessels in incubator at 37°C and 5% CO_2. • Feed cells every 2-3 days by completely replacing the medium with fresh 37°C warm I-GRO medium with TGF-β-3 added fresh each time. Observe cells daily • Once cells have become 80% confluent, prepare cells for passage, expansion, or freezing.
4.	Passaging of 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. • Wash cell culture vessel with equal volume of PBS to obtain any remaining cells. Add this PBS wash from the cell culture vessel to the centrifuge tube. • Centrifuge the cell suspension at 400 g at 4°C for 5 min. Aspirate supernatant.

- Disperse cell pellet in 37°C warm I-GRO medium with TGF-β-3 added fresh. Take sample of this cell suspension for counting.
- Count cells (see How to Count Fresh Cells).
- To propagate cells, seed cells in culture vessel at a density of 3.0-5.0 x 10^3cells/cm^2 in I-GRO medium with TGF-β-3.
- If more cells are obtained than needed for re-plating, the additional cells may be frozen. (See How to Freeze Cells).

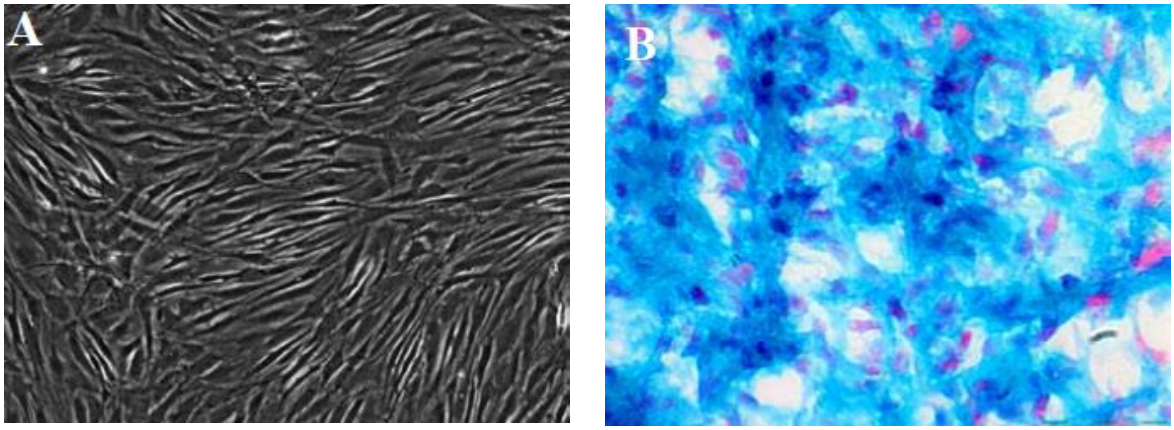

FIGURE 1: Cells were grown in Fibroblasts Cellutions media with the addition of growth factor TGF-β-3. **(A)** AM006-F, passage 2, phase contrast. **(B)** AM006-F, stained with alcian blue (proteoglycans), counter-stained with nuclear fast red.