Materials and Equipment

Materials

- Valvular Interstitial Cells (AC024-F)
- Fibroblast Cellutions PLUS Medium (I-GRO PLUS)adding 20ng/ml of FGF-2
- 1× PBS (Ca^{2+}-/Mg^{2+}-free)
- Dulbecco's Modified Eagle Medium (DMEM)
- Fetal Bovine Serum (FBS)
- Trypsin solution (0.05% trypsin, 0.02% EDTA) or equivalent
- 70% isopropyl alcohol (IPA)
- 50ml tubes

Equipment

- Incubator, 37°C/ 5% CO_2.
- Tissue culture vessels
- Water Bath, 37 °C
- Centrifuge
- Pipettes

Procedure

1.	Adopt aseptic practices for all steps involving cell culture.
2.	Remove cryovial from shipping container to check that it is still frozen—if thawed contact DV Biologics. Transfer frozen vial to liquid nitrogen until you are ready to thaw and begin cell culture.
3.	Thaw cryovial in 37°C water bath with agitation. For details see How to Thaw Cells.
4.	Transfer contents (1 to 2ml) of cryovial to 50ml tube. Slowly add dropwise with agitation 9ml FBS (pre-warmed at 37°C).
5.	Centrifuge 50-ml conical tube at 400g for 5 minutes at 4°C.
6.	Remove supernatant; disperse cell pellet with I-GRO PLUS; add sufficient I-GRO PLUS to make 1 to 5ml; determine cell count. For details see How to Count Cells.
7.	Seed cells at densities 1.0 × 10^4 cells/ cm^2 in I-GRO-PLUS; incubate at 37°C, 5% CO_2; feed cells every 2 to 3 days.
8.	When cells are 80 to 90% confluent, they can be subcultured.
9.	To subculture, remove spent medium; rinse cells twice with PBS (pre-warmed at 37°C); dissociate cells with TrypLE Express solution (37°C, 5 to 10min); quench dissociation with I-Gro PLUS; centrifuge cells at 400g at 4°C for 5 min.
10.	Remove supernatant; disperse cell pellet with I-GRO PLUS; add sufficient I-GRO PLUS to make 1 to 5ml; determine cell count.
11.	Seed cells at densities 1.0 × 10^4 cells/ cm^2 in I-GRO PLUS; incubate at 37°C, 5% CO_2; replace spent medium with fresh medium every 2 to 3 days or alternatively freeze cells. For details see How to Freeze Cells.