Materials and Equipment

Materials

- Adrenal Gland Cells (Uncultured) (AE006-F)
- Fibroblast Cellutions Medium (I-GRO)
- 1× PBS (Ca^{2+}-/Mg^{2+}-free)
- Dulbecco's Modified Eagle Medium (DMEM)
- Fetal Bovine Serum (FBS)
- TrypLE Express
- 70% isopropyl alcohol (IPA)
- 50ml Tubes

Equipment

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

Procedure

1.	Immediately upon delivery • Remove vial 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.
2.	Thawing Cells • Perform and maintain all cell culture using aseptic techniques • Prepare I-GRO medium. • Mix and store any unused medium in 4°C for a maximum of 30 days. • Warm necessary media prior to commencing to 37°C. • Thaw cells rapidly and with agitation (See How to Thaw Cells). • Count cells (see How to Count Fresh Cells).
3.	Plating and Maintaining Cells • Carefully add cell suspension to culture vessel. Seed cells at a density of 1.0 x 10^4 cells/cm^2. • Observe cells on a daily basis. Note: It will appear to be a lot of debris. These are cells and clumps of cells that will attach and grow. Do not remove or aspirate the cells until after 4 day media change • Feed cells after 4 days by performing a complete media change. • Once cells become 80-90% confluent cells are ready for testing compounds Note: if testing media (excretion of cells), leave media on cells undisturbed for no less than 1 day
4.	Passaging of Cells (for Details see How to Passage Cells). • Aspirate and discard medium; wash cells with warm (37°C) PBS, aspirate, and add enzyme solution. • Incubate for 5 min at 37°C. NOTE: Observe detachment of cells. When using trypsin observe the detachment of cells every 1-2 minutes in order to avoid toxic effects. • Add 10% complete medium or FBS immediately to detached cell suspension to stop reaction. Transfer cell suspension to a desired centrifuge tube. • Wash cell culture vessel by adding equal volume (to enzyme amount used) warm PBS to the culture vessel to ensure that you have obtained all cells. Add the washed material to centrifuge tube • Centrifuge your cell suspension at 400 g at 4°C for 5 min. • Aspirate supernatant and resuspend cells in fresh medium for counting Note: for in vitro testing passaged cells may not function like primary cells • If more cells are obtained than needed for replating, freeze additional cells. (See How to Freeze Cells).