Scope

This culture guide describes the in vitro culture of normal human prenatal whole large intestine cells using DV Biologics’ cells, media, and solutions. These cells are comprised of multicellular aggregates rather than a single cell suspension. Any changes in the experimental conditions may have negative effects on cell survival and may yield abnormal cell growth.

Materials and Equipment

Materials

- Large Intestine (uncultured) Cells (PD008-F)
- Cell Dissociation Solution (CCS101)
- Culture Vessel Coating Solution (CCS102)
- Pro-Conditioned Epithelial Cell Medium (D-PRO)
- 1× PBS (Ca^{2+}/Mg^{2+}-free)
- 15ml & 50ml tubes
- Dimethyl Sulfoxide (DMSO)
- Dulbecco’s modified Eagle medium (DMEM)
- Fetal Bovine Serum (FBS)
- Ice

Equipment

- Cell culture vessels
- Centrifuge
- Cryovials
- Incubator, 37°C, 5% CO_{2}
- Pipette
- Water Bath, 37°C

Procedure

1. Upon receipt
   - 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. Coating—prior to thawing cells
   - Thaw CCS102 at 4°C for 2-3 hours.
   - Warm CCS102 solution to room temperature prior to use.
   - Add CCS102 to the desired cell culture vessel as suggested in the table below.
   - NOTE: Be sure that solution covers the coating surface entirely

<table>
<thead>
<tr>
<th>Cell Culture Vessel</th>
<th>Medium Volume (ml)</th>
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</thead>
<tbody>
<tr>
<td>12-well</td>
<td>0.4</td>
</tr>
<tr>
<td>6-well</td>
<td>1.0</td>
</tr>
<tr>
<td>T25</td>
<td>2.5</td>
</tr>
<tr>
<td>T75</td>
<td>7.5</td>
</tr>
</tbody>
</table>
   - Incubate at room temperature, 1h.
   - Remove coating solution by aspiration and discard.
   - Wash culture vessel with PBS.

3. Thawing Cells (For details see How to Thaw Cells)
   - Thaw cryovial in 37°C water bath with agitation.
   - Transfer contents to 50ml tube.
   - Slowly and drop-wise add 9ml of FBS to the cells with agitation.
   - Centrifuge to pellet at 400g at 4°C for 5min.
   - Remove supernatant and resuspend cell pellet in warm D-PRO; determine cell count (see How to Count Cells).
   - NOTE: Cells are provided as multi-cellular aggregates, thus the conventional method of cell counting often underestimates the actual cell count.
   - Keep cells on ice
4. Cultivation

- Calculate the number of cells needed to obtain a $5 \times 10^4$ cells/cm$^2$ seeding density; prepare a corresponding cell suspension (see table below), using D-PRO medium.

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</table>

- Add cell suspension to the cell culture vessel.
- Incubate at 37°C, 5% CO$_2$.
  NOTE: Cell clusters take at least 24 hours to fully adhere to the growth surface.
- Replace medium after 24 hours in order to remove cell debris, then every 2 or 3 days.
  NOTE: To maximize the number of serial passages detach cells (Section 5) at 80% confluency

5. Detaching Cells

- Thaw CCS101 at 37°C with agitation (avoid passive thaw).
- Remove medium; wash cells with PBS.
- Add CCS101 (use same volume as suggested for medium (Section 4); incubate for 20 min at 37°C.
- Agitate culture vessel and transfer detached cell suspension into a 50ml conical tube containing two volumes of cold DMEM + 10% FBS; set tube on ice.
- Add another volume of CCS101 to the vessel and incubate 37°C, 20min; agitate culture vessel and pool detached cell suspension with the previously collected fraction.
- Wash culture vessel with one volume of PBS and pool with the previously collected fraction.
  NOTE: Detachment procedure yields multi-cellular aggregates, which is normal.
- Centrifuge the collected cell suspension 400g at 4°C for 5 min; aspirate supernatant
- Proceed with sub-cultivation (Section 6) or freezing (Section 7).

6. Sub-cultivation

NOTE: The morphology of intestinal cells may change after its passage.
- Warm CCS101 and D-PRO medium to room temperature.
- Select a culture vessel; coat the vessel with Culture Vessel Coating Matrix solution (Section 4).
- Resuspend cell pellet (from Section 5) in D-PRO medium.
- Select Culture Vessel.
- Count Cells (see How to Count Cells).
- Refer to Section 3 from seeding density and prepare cell suspension using D-PRO; transfer the cell suspension to the vessel.
- Incubate culture vessel 37°C, 5% CO$_2$, replacing medium every 2 to 3 days.

7. Freezing (See How to Freeze Cells).