Primary Epithelial Cell Culture Protocol

- All cell culture procedures must be conducted in bio-safety cabinet.
- Any and all media, supplements, and reagents must be sterilized by filtration through a 0.2 µm filter.
- Use aseptic technique to prevent microbial contamination.
- Cryo-preserved cells must be stored in liquid nitrogen or seeded immediately upon arrival.

Medium

- Review the information provided on the Cell Biologics website for appropriate complete culture media (e.g. serum and other supplements).
- Only pre-warm (37°C) the amount of cell culture media you will be using right away. 5 ML for a T25 or 10 ML for a T75 flask is needed when recovering cryo-preserved cells, changing media or splitting cells.
- Repeated warming of complete culture media is not recommended.

Coating of Flasks or Dishes

Coat sterile culture dishes or flasks with Gelatin-Based Coating Solution (Cell Biologics, Catalog No. 6950) for 2 min and then aspirate the excess solution before seeding cells.

Handling of Live Cells upon arrival

- Upon receiving the live cells in a T25 or T75 flask, remove the sticker from the filter cap, place the flask with 6-20 ml of the existing medium into 37°C, 5% CO_2 incubator for 1 hour, then replace the medium with desired Cell Biologics' cell culture medium.
- These cells at 90% confluence can be split at 1:2 ratio for subculturing or frozen down for future usage when 95-100% confluent.

Cell Recovery from Cryovial

1. Quickly thaw cells by placing the vial in a 37°C water bath for <1 min or until there is just a small bit of ice left in the vial.
2. Promptly remove the vial and wipe it down with 70% ethanol.
3. Transfer cells from the vial to a 15 ml sterile conical centrifuge tube with 5-6 ml of pre-warmed Cell Biologics Cell Culture Medium.
4. Flush the vial with an additional 0.5-1 ml of medium to collect additional cells left in the vial.
5. Centrifuge the cells at 150 x g for 5 minutes. Note: Centrifugation of cells after thawing to remove residual DMSO is recommended.
6. Carefully aspirate the supernatant and resuspend the cell pellet with 5 ml of Cell Biologics' Cell Culture Growth Medium. Add resuspended cells into a T25 flask pre-coated with Gelatin-Based Coating Solution (Cell Biologics, Catalog No. 6950).
7. Place the seeded T25 flask in a humidified, 5% CO_2 incubator at 37°C. Leave the culture undisturbed for at least 16 hours.
8. Change culture media the following day and then every 24-48 hours afterwards to remove non-adherent cells and replenish nutrients. Please note that the medium should be changed every day when cells are >70-80% confluent.
9. Cells should be checked daily under a microscope for cell confluence and appropriate cell morphology.

Expansion of Cultured Primary Cells

1. Remove and discard the cell culture media from the flask.
2. Flush the adherent cell layer 1-2 times with sterile 1X PBS (without calcium and magnesium) to remove residual medium.
3. Incubate the cells with pre-warmed (37°C) 0.05% Trypsin-EDTA solution (Cell Biologics, Catalog No. 6915) for 3-5 minutes. Use 3.0 ml of Trypsin-EDTA solution for a T75 flask and 2 ml for a T25 flask, respectively. As soon as the cells have detached (a few firm gentle taps on side of flask may help detachment), add 5 ml

of Cell Biologics' Cell Culture Medium to the flask (the FBS will neutralize the trypsin) and gently pipette up and down a few times.

4. Seed the cells in fresh flasks/plates precoated with Gelatin, and then return flasks/plates into a humidified, 37°C, 5% CO_2 incubator.
5. Change culture media the following day and then every 24-48 hours afterwards to remove non-adherent cells and replenish nutrients. Please note that the medium should be changed every day when cells are >70-80% confluent.
6. Cells should be checked daily under a microscope for cell confluence and appropriate cell morphology.
7. Cells can be pre-washed with 1X PBS 1-2 times whenever replacing the medium (optional).

We recommend splitting primary cells at the following ratio

- The recommended split ratio for primary cells is 1:2.

Procedure for Freezing Cells

Materials:

1. 1X Phosphate Buffered Saline (PBS-1X)
2. 0.05% Trypsin-EDTA (1X) solution (Cell Biologics, Catalog No. 6915)
3. Tissue Culture Media
4. Cold Freezing Media (10% DMSO, 50% FBS and 40% culture medium, Cell Biologics, Catalog No. 6916).
5. Labeled Cryovials
6. Confluent Cells

---

1. Remove and discard the cell culture media from the flask.
2. Flush the adherent layer 2 times with 7 ml sterile PBS (1X, without calcium and magnesium) to remove residual medium.
3. Incubate the cells with pre-warmed (37°C) 0.05% Trypsin-EDTA solution (Cell Biologics, Catalog No. 6915) for 3-5 minutes. Use 3.0 ml of Trypsin-EDTA solution for a T75 flask and 2 ml for a T25 flask, respectively. As soon as the cells have detached (a few firm gentle taps on side of flask may help detachment), add 5 ml of Cell Biologics' Cell Culture Medium to the flask (the FBS will neutralize the trypsin) and gently pipette up and down 1-2 times.
4. Transfer the cells suspension to a centrifuge tube, centrifuge cells at 150 x g for 5 minutes.
5. Carefully remove supernatant with sterile Pasteur pipette.
6. Quickly re-suspend cell pellet by adding 1 ml freezing media per vial to be frozen.
7. Place vials in Nalgene "Mr. Frosty" freezing container containing100% isopropyl alcohol at -80 °C for 12h.
8. Transfer vials to liquid nitrogen tank for indefinite storage.

We recommend freezing primary cells at the following ratio

- A confluent primary epithelial cells grown in a T75 flask may be frozen in 2-3 cryovials.
- A confluent primary epithelial cells grown in a T25 flask may be frozen in 1 cryovial.
- Please send us light microscope cell images (Cell confluence >70-80%) if you have any questions with cultured cells.