

**SOP:** Propagation of human retinal pigment epithelial cells (HRPEpiC, ScienCell)  
**Date modified:** 12/11/09  
**Modified by:** E. Giste/T. Canfield (UW)

### **Ordering Information**

Human retinal pigment epithelial cells (HRPEpiC) may be ordered as frozen ampoules. Each vial contains  $>5 \times 10^5$  cells in a 1ml volume.

To order frozen ampoules + media:

Name:	HRPEpiC – Human Retinal Pigment Epithelial Cells
Item #:	6540 (HRPEpiC - Cryopreserved ampoule)
	4101 (EpiCM, Epithelial Cell Medium)

### **Notes:**

The number of media kits purchased depends on the target number of cells to be generated. A rule of thumb is 10 media kits for every initial cryopreserved ampoule. It is strongly recommended to purchase all of the media that will be required for a complete expansion series, since media supply may be erratic. All culture flasks must be coated with poly-L-lysine ( $2\mu\text{g}/\text{cm}^2$ ).

### **Materials List**

1. EpiCM Epithelial Cell Medium (ScienCell, Cat# 4101)
2. Poly-L-Lysine (10mg/ml, ScienCell, Cat# 0413)
3. Phosphate Buffered Saline (1X PBS) (prepared from 10X stock Cellgro, Cat# 46-013-CM by dilution with sterile deionized water)
4. Accutase Enzyme Cell Detachment Medium (EBioscience, Cat# 00-4555)
5. T225 culture flasks
6. Graduated pipets (1, 5, 10, 25, 50mL)
7. Pen-Strep solution (if required; ScienCell typically supplies antibiotics)
8. Hemocytometer
9. Micropipet w/ P20 tips
10. Microscope

### **Procedure**

#### **A. Initiation of culture from cryopreserved cells**

- 1) Rapidly thaw cells by holding vial and gently rotating in  $37^\circ\text{C}$  water bath.
- 2) As soon as ice crystals disappear, dispense contents of vial into a flask at  $5,000 \text{ cells}/\text{cm}^2$  density.
- 3) Let cells recover for 16 hours in a  $37^\circ\text{C}$ , 5%  $\text{CO}_2$  humidified incubator.
- 4) The next morning, the diluted DMSO-containing shipping/cryopreservation medium is aspirated from the cell layer and replaced with fresh medium.

#### **B. Sub-culture**

- 1) Propagate cells until density reaches 90% confluence.
- 2) Aspirate medium.
- 3) Wash cells with warm 1X PBS.
- 4) Add 15mLs of Accutase and return to incubator for 10-15 minutes, or until cells detach.
- 5) Immediately remove cells, rinse flask with warm 1X PBS to collect residual cells, and pellet at 500 x g for 5 minutes (4°C).
- 6) Gently re-suspend cell pellet in warm medium.
- 7) Count cells with hemocytometer.
- 8) Add warmed medium to flasks.
- 9) Seed flasks at 5,000 cells/cm<sup>2</sup>
- 10) Record each subculture event as a passage.

### **C. Maintenance**

- 1) Change media the day after seeding and every OTHER day thereafter.
- 2) Increase media volume as confluency increases (volumes assume the use of
- 3) T225 flasks):
  - a. 25 % = 1mL/5 cm<sup>2</sup>
  - b. 25-45% = 1.5mL/ 5 cm<sup>2</sup>
  - c. 45%+ = 2mL/ 5 cm<sup>2</sup>.
- 4) Per the above an exemplary schedule might be:
  - a. day 1, plate into T225: use 50 mls of media.
  - b. day 2, change media, use 50 mls of media
  - c. day 4, change media, use 100 mls of media (if confluency is >50%)
  - d. day 6, change media, use 100 mls of media (or harvest if ready)
  - e. day 7 or 8 (harvest when cells reach 6 x 10<sup>6</sup> cells/flask

### **D. Harvest**

- 1) Pass cells 3-4 times until the desired cell number is achieved (primary cells will senesce after 4-5 passages).
- 2) Remove cells from flasks according to protocol described above under 'Sub-culture'.
- 3) Examine viability using Trypan blue staining (SOP TP-7).