

## Instruction Manual

Product	Size	Catalog Number
Human Nasal Epithelial Cells (HNEpC)	500,000 cryopreserved cells 500,000 proliferating cells	C-12620 C-12621
Human Tracheal Epithelial Cells (HTEpC)	500,000 cryopreserved cells 500,000 proliferating cells	C-12644 C-12645
Human Bronchial Epithelial Cells (HBEpC)	500,000 cryopreserved cells 500,000 proliferating cells	C-12640 C-12641
Human Small Airway Epithelial Cells (HSAEpC)	500,000 cryopreserved cells 500,000 proliferating cells	C-12642 C-12643
Human Mammary Epithelial Cells (HMEpC)	500,000 cryopreserved cells 500,000 proliferating cells	C-12650 C-12651
Human Renal Epithelial Cells (HREpC)	500,000 cryopreserved cells 500,000 proliferating cells	C-12665 C-12666
Human Renal Cortical Epithelial Cells (HRCEpC)	500,000 cryopreserved cells 500,000 proliferating cells	C-12660 C-12662
Human Placental Epithelial Cells (HPIEpC)	500,000 cryopreserved cells 500,000 proliferating cells	C-12395 C-12397

### Product Description

The epithelium is the interface between the body and the external environment and covers all exterior surfaces and interior lumina. Depending on the tissue of origin, the functions of epithelial cells are diverse and include absorption, secretion, protection, transcellular transport, and sensation.

PromoCell offers a range of Epithelial Cells produced at PromoCell's cell culture facility. The cells are isolated from normal human adult tissues of the airways (nasal mucosa, trachea, bronchi, and distal respiratory tract), breast (mammary gland), kidney (whole kidney and renal cortex), and the placenta (amniotic membrane).

Shortly after isolation, all PromoCell Human Epithelial Cells are cryopreserved at passage 2 (P2) using PromoCell's proprietary, serum-free freezing medium, Cryo-SFM. Each cryo vial contains more than 500,000 viable cells after thawing.

Proliferating cell cultures are made from 500,000 cryopreserved cells that have been thawed and cultured for three days at PromoCell.

### Quality Control

Rigid quality control tests are performed for each lot of Epithelial Cells.

They are tested for cell morphology, adherence rate and cell viability. Furthermore, immunohistochemical tests for the cell-type specific marker cytokeratin are carried out for each lot (see page 5). Growth performance is tested through multiple passages under culture conditions without antibiotics and antimycotics. In addition, all cells have been tested for the absence of HIV-1, HIV-2, HBV, HCV, and microbial contaminants (fungi, bacteria, and mycoplasma).

A detailed certificate of analysis (CoA) for each lot can be downloaded at: [www.promocell.com/coa](http://www.promocell.com/coa)

### Intended Use

PromoCell Epithelial Cells are for *in vitro* research use only and not for diagnostic or therapeutic procedures.

### Warning

Although tested negative for HIV-1, HIV-2, HBV, and HCV, the cells - like all products of human origin - should be handled as potentially infectious. No test procedure can completely guarantee the absence of infectious agents.

**Follow appropriate safety precautions!**

*After delivery, start immediately with the protocol for cryopreserved cells (see page 2) or the protocol for proliferating cells (see page 3).*

*Start immediately after delivery.  
Use aseptic techniques and a laminar flow bench.*

### Protocol for Cryopreserved Cells

**Straight after arrival, store the cryopreserved cells in liquid nitrogen, or seed them immediately.**

**Note:** Storage at  $-80^{\circ}\text{C}$  is not sufficient for cell preservation and causes irreversible cell damage.

#### 1. Prepare the medium

Calculate the needed culture surface area according to the plating density (see page 5). Fill the appropriate volume of PromoCell Growth Medium (at least 9 ml per vial of cells) in cell culture vessels. Place the vessels in an incubator ( $37^{\circ}\text{C}$ , 5%  $\text{CO}_2$ ) for 30 minutes.



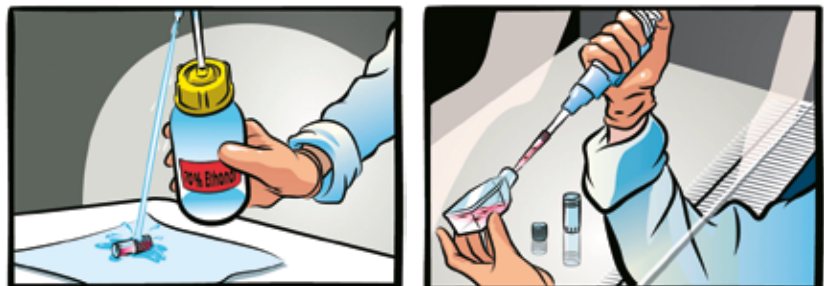
#### 2. Thaw the cells

Remove the cryovial from the liquid nitrogen container and immediately place it on dry ice - even for short transportation. Under a laminar flow bench, briefly twist the cap a quarter turn to relieve pressure, then re-tighten. Immerse the vial into a water bath ( $37^{\circ}\text{C}$ ) just up to the screw cap for 2 minutes. Ensure that no water enters the thread of the screw cap.



#### 3. Disinfect the vial and seed the cells

Thoroughly rinse the cryovial with 70% ethanol under a laminar flow bench. Then, aspirate the excess ethanol from the thread area of the screw cap. Open the vial and transfer the cells to a cell culture vessel containing the prewarmed medium from step 1.



#### 4. Incubate the cells

Place the vessel in an incubator ( $37^{\circ}\text{C}$ , 5%  $\text{CO}_2$ ) for cell attachment. Replace the medium after 16 - 24 hours. The cells should be subcultured, according to the subcultivation protocol (see page 4), once they have reached 70 - 90% confluency.



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### Protocol for Proliferating Cells

#### 1. Incubate the cells

Unpack the culture vessel, do not open the lid, and immediately place it in an incubator (37°C, 5% CO<sub>2</sub>) for 3 hours.



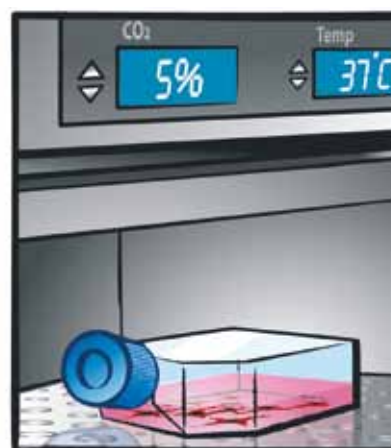
#### 2. Replace the transport medium

Carefully open the vessel, rinse the inner side of the lid with 70% ethanol, and let air dry. Aspirate the transport medium from the vessel. Add 10 ml of the appropriate PromoCell Cell Growth Medium.



#### 3. Check and incubate the cells

Check the cell density. Open the lid a half turn and place the vessel in an incubator (37°C, 5% CO<sub>2</sub>). The cells should be subcultured, according to the subcultivation protocol (see page 4), once they have reached 70 – 90% confluency.





*Use aseptic techniques and a laminar flow bench.*

## Subcultivation Protocol

### 1. Prepare the reagents and wash the cells

Place the PromCell DetachKit at room temperature for at least 30 minutes to adjust the temperature of the reagents. Carefully aspirate the medium from the culture vessel. Add 100  $\mu$ l Hepes BSS Solution per  $\text{cm}^2$  of vessel surface to wash the cells and agitate the vessel carefully for 15 seconds.



### 2. Detach the cells

Carefully aspirate the Hepes BSS from the culture vessel. Add 100  $\mu$ l Trypsin/EDTA Solution per  $\text{cm}^2$  of vessel surface. Note: We recommend detaching the cells at room temperature. Close the vessel and examine the cells under a microscope. When the cells start to detach, gently tap the side of the vessel to loosen the remaining cells.



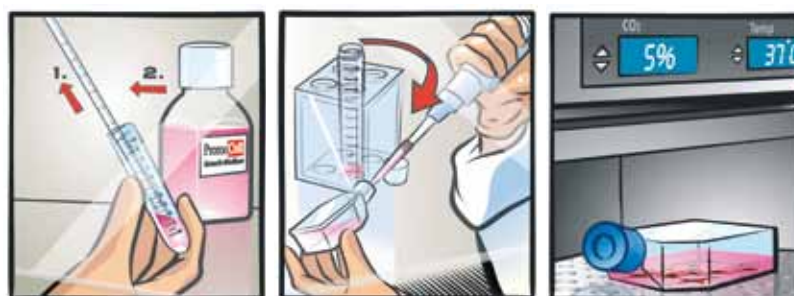
### 3. Neutralize the trypsin and harvest the cells

Add 100  $\mu$ l Trypsin Neutralization Solution per  $\text{cm}^2$  of vessel surface and gently agitate. Carefully aspirate the cell suspension and transfer it to a centrifugation tube. Spin down the cells for 3 minutes at 220 x g.



### 4. Incubate the cells

Discard the supernatant (step 1), add 1 ml of the appropriate PromoCell Cell Growth Medium (step 2), and resuspend the cells by carefully pipetting up and down. Plate the cells according to the recommended seeding density in new cell culture vessels containing PromoCell Cell Growth Medium prewarmed to 37°C. Place the vessels in an incubator (37°C, 5%  $\text{CO}_2$ ).



## Specifications

Product	Recommended Culture Media*	Plating Density	Passage after Thawing	Marker	Population Doublings
Human Nasal Epithelial Cells (HNEpC)	C-21060	10,000 - 15,000 cells per cm <sup>2</sup>	P2	Cytokeratin <sup>+</sup>	> 15
Human Tracheal Epithelial Cells (HTEpC)	C-21060	10,000 - 15,000 cells per cm <sup>2</sup>	P2	Cytokeratin <sup>+</sup>	> 15
Human Bronchial Epithelial Cells (HBEpC)	C-21060	10,000 - 15,000 cells per cm <sup>2</sup>	P2	Cytokeratin <sup>+</sup>	> 15
Human Small Airway Epithelial Cells (HSAEpC)	C-21070	10,000 - 15,000 cells per cm <sup>2</sup>	P2	Cytokeratin <sup>+</sup>	> 15
Human Mammary Epithelial Cells (HMEpC)	C-21010	10,000 - 15,000 cells per cm <sup>2</sup>	P2	Cytokeratin <sup>+</sup>	> 15
Human Renal Epithelial Cells (HREpC)	C-26001 C-26030	10,000 - 15,000 cells per cm <sup>2</sup>	P2	Cytokeratin <sup>+</sup>	> 15
Human Renal Cortical Epithelial Cells (HRCepC)	C-26001 C-26030	10,000 - 15,000 cells per cm <sup>2</sup>	P2	Cytokeratin <sup>+</sup>	> 15
Human Placental Epithelial Cells (HPIEpC)	C-26040	10,000 - 15,000 cells per cm <sup>2</sup>	P2	Cytokeratin <sup>+</sup>	> 5

## Related Products

Product	Size	Catalog Number
Airway Epithelial Cell Growth Medium (Ready-to-use)	500 ml	C-21060
Airway Epithelial Cell Growth Medium Kit	500 ml	C-21160
Airway Epithelial Cell Basal Medium	500 ml	C-21260
Airway Epithelial Cell Basal Medium, phenol red-free	500 ml	C-21265
Airway Epithelial Cell Growth Medium SupplementMix	for 500 ml	C-39165
Airway Epithelial Cell Growth Medium SupplementPack	for 500 ml	C-39160
Small Airway Epithelial Cell Growth Medium (Ready-to-use)	500 ml	C-21070
Small Airway Epithelial Cell Growth Medium Kit	500 ml	C-21170
Small Airway Epithelial Cell Basal Medium	500 ml	C-21270
Small Airway Epithelial Cell Basal Medium, phenol red-free	500 ml	C-21275
Small Airway Epithelial Cell Growth Medium SupplementMix	for 500 ml	C-39175
Small Airway Epithelial Cell Growth Medium SupplementPack	for 500 ml	C-39170
Mammary Epithelial Cell Growth Medium (Ready-to-use)	500 ml	C-21010
Mammary Epithelial Cell Growth Medium Kit	500 ml	C-21110
Mammary Epithelial Cell Basal Medium	500 ml	C-21210
Mammary Epithelial Cell Basal Medium, phenol red-free	500 ml	C-21215
Mammary Epithelial Cell Growth Medium SupplementMix	for 500 ml	C-39115
Mammary Epithelial Cell Growth Medium SupplementPack	for 500 ml	C-39110

\*The catalog numbers in this table are for media in ready-to-use packaging.

Product	Size	Catalog Number
Renal Epithelial Cell Growth Medium (Ready-to-use)	500 ml	C-26001
Renal Epithelial Cell Growth Medium Kit	500 ml	C-26011
Renal Epithelial Cell Basal Medium	500 ml	C-26021
Renal Epithelial Cell Basal Medium, phenol red-free	500 ml	C-26022
Renal Epithelial Cell Growth Medium SupplementMix	for 500 ml	C-39604
Renal Epithelial Cell Growth Medium SupplementPack	for 500 ml	C-39600
Renal Epithelial Cell Growth Medium 2 (Ready-to-use)	500 ml	C-26030
Renal Epithelial Cell Growth Medium 2 Kit	500 ml	C-26130
Renal Epithelial Cell Basal Medium 2	500 ml	C-26230
Renal Epithelial Cell Basal Medium 2, phenol red-free	500 ml	C-26235
Renal Epithelial Cell Growth Medium 2 SupplementMix	for 500 ml	C-39606
Renal Epithelial Cell Growth Medium 2 SupplementPack	for 500 ml	C-39605
Placental Epithelial Cell Growth Medium (Ready-to-use)	500 ml	C-26040
Placental Epithelial Cell Growth Medium Kit	500 ml	C-26140
Placental Epithelial Cell Basal Medium	500 ml	C-26240
Placental Epithelial Cell Basal Medium, phenol red-free	500 ml	C-26245
Placental Epithelial Cell Growth Medium SupplementMix	for 500 ml	C-39612
Placental Epithelial Cell Growth Medium SupplementPack	for 500 ml	C-39610
DetachKit	30 ml 125 ml 250 ml	C-41200 C-41210 C-41220
Cryo-SFM	30 ml 125 ml	C-29910 C-29912
HNEpC Pellet	1 million cells per pellet	C-14062
HTepC Pellet	1 million cells per pellet	C-14064
HBEpC Pellet	1 million cells per pellet	C-14063
HSAEpC Pellet	1 million cells per pellet	C-14065
HMEpC Pellet	1 million cells per pellet	C-14066
HREpC Pellet	1 million cells per pellet	C-14067
HRCEpC Pellet	1 million cells per pellet	C-14068
HPIEpC Pellet	1 million cells per pellet	C-14069
BPE-15	2 ml	C-30020
BPE-26	2 ml	C-30021
EGF, human, recombinant	500 µg	C-60170

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