Airway epithelial cells

PromoCell®

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

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.

We offer airway epithelial cells isolated from normal human adult tissues of the nasal mucosa (HNEpC), trachea (HTEpC), bronchi (HBEpC), and distal respiratory tract (HSAEpC).

Shortly after isolation, all our airway epithelial cells are cryopreserved using our proprietary, animal-component free, and protein-free cryopreservation medium (for details, please refer to the Certificate of Analysis).

Each cryovial contains more than 500,000 viable cells after thawing. Proliferating cell cultures are made from cryopreserved cells that have been thawed and cultured for three days in our hands and shipped as growing cultures.

Quality control

We perform rigid quality control tests for each lot of airway epithelial cells.

The cells are tested for cell morphology, adherence rate and cell viability. Furthermore, flow cytometric analyses 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 or antimycotics. Upon request we provide HBEpC donors tested for proper epithelial cell barrier function in our Air-Liquid Interface (ALI) Medium (C-21080). ALI pre-screened HBEpC are tested by voltohmmeter for a stable transepithelial electrical resistance (TEER) of >500 Ω*cm^2.

In addition, all cells have been tested for the absence of HIV-1, HIV-2, HBV, HCV, HTLV-1, HTLV-2 and microbial contaminants (fungi, bacteria, and mycoplasma).

Intended use

Our airway 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, HCV, HTLV-1 and HTLV-2, 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, cryopreserved cells should be stored in liquid nitrogen or seeded directly (see page 2). Proliferating cells must be processed immediately (see page 3).

Protocol for cryopreserved cells

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

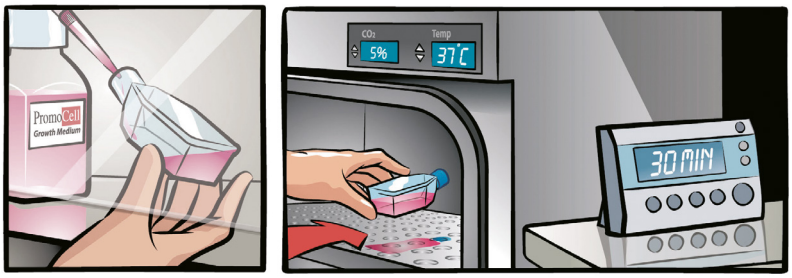
Note: Storage at -80°C is not sufficient for cell preservation and causes irreversible cell damage.

Use aseptic techniques and a laminar flow bench.

1

Prepare the medium

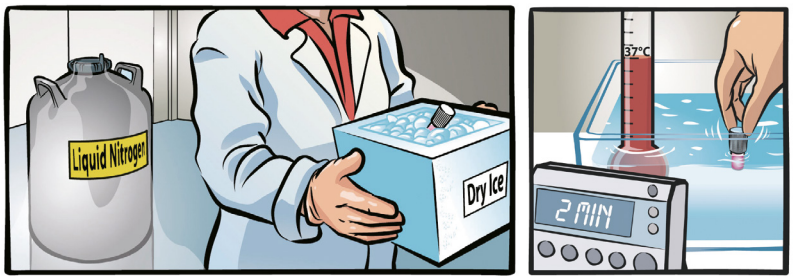
Calculate the required culture surface area according to the plating density (see page 5) and the lot-specific cell numbers stated on the certificate of analysis. Fill the appropriate volume of PromoCell Growth Medium (at least 9 ml per vial of cells) in cell culture vessels. Place the vessels in an incubator (37°C, 5% 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 retighten. Immerse the vial in a water bath (37°C) up to the height of the screw cap for 2 minutes. Ensure that no water enters the thread of the screw cap.

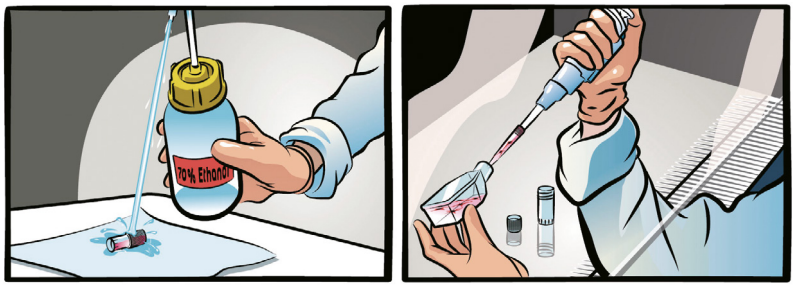


3

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 pre-warmed medium from step 1.

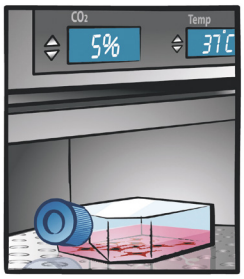
Note: When culturing airway epithelial cells in Airway Epithelial Cell Growth Medium 2 (C-21040) or Airway Epithelial Cell Growth Medium XF (prf) (C-21050), we recommend centrifuging the cells prior to seeding to remove any residual DMSO. To do this, centrifuge the cells at 300 × g for 3 minutes, then carefully aspirate the supernatant. Resuspend the cell pellet in a small volume of medium, and calculate the volume required for seeding. Finally, seed the cells into a culture vessel containing pre-equilibrated growth medium.



4

Incubate the cells

Place the vessel in an incubator (37°C, 5% CO_2) for cell attachment. Replace the medium after 16–24 hours and every two to three days thereafter. The cells should be subcultured, according to the subcultivation protocol (see page 4), once they have reached 70–90% confluency.





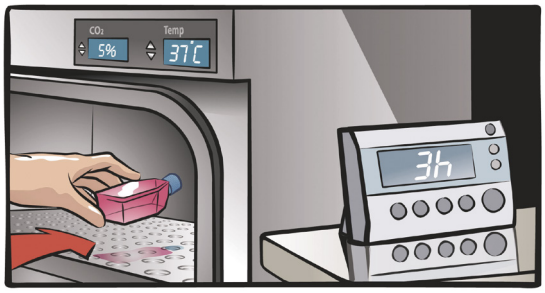
Protocol for proliferating cells

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

1

Incubate the cells

Unpack the culture vessel, do not open the cap, and immediately place it in an incubator (37°C, 5% CO_2) for 3 hours to allow the cells to recover from transportation.



2

Replace the transport medium

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



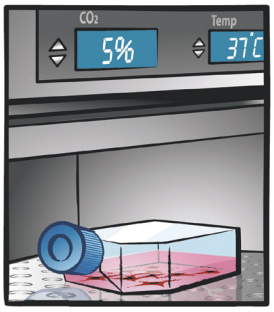


3

Check and incubate the cells

Check the cell density. Open the lid half a turn and place the vessel in an incubator (37°C, 5% CO_2). Change the medium every two to three days. The cells should be subcultured, according to the subcultivation protocol (see page 4), once they have reached >70% confluency.





Product	Size	Catalog Number
DetachKit	30 ml 125 ml 250 ml	C-41200 C-41210 C-41220
Cryo-SFM Plus	30 ml 125 ml	C-29920 C-29922
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
BPE-15	2 ml	C-30020
BPE-26	2 ml	C-30021

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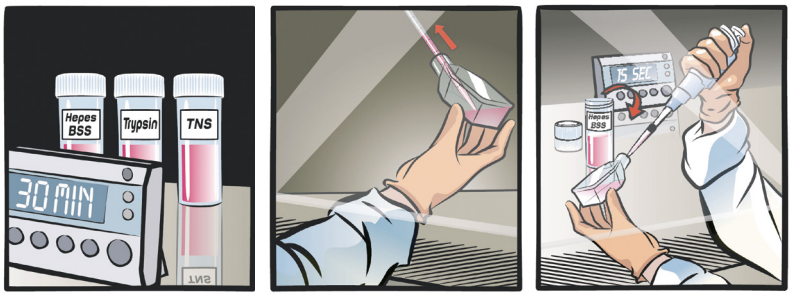
Subcultivation protocol

Use aseptic techniques and a laminar flow bench.

1

Prepare the reagents and wash the cells

Place the PromoCell 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 µl Hepes BSS Solution per cm^2 of vessel surface to wash the cells and agitate the vessel carefully for 15 seconds.

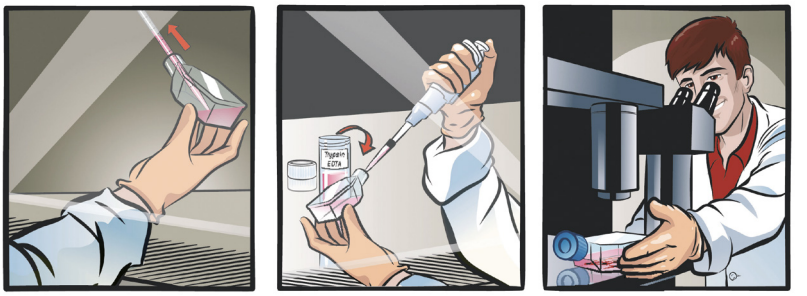


2

Detach the cells

Carefully aspirate the Hepes BSS from the culture vessel. Add 100 µl Trypsin/EDTA Solution per cm^2 of vessel surface.

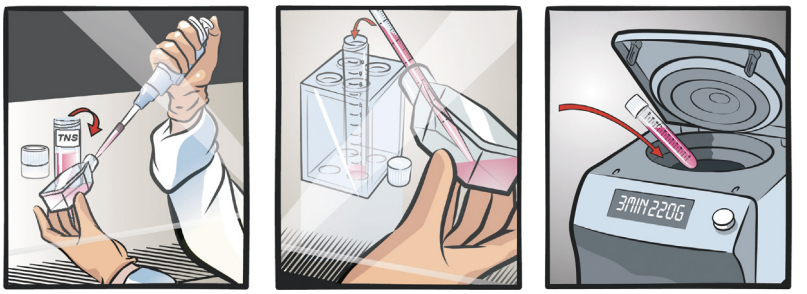
Note: We recommend detaching the cells at 37°C for 4 minutes. Close the vessel and examine the cells under a microscope. When the cells start to detach, gently tap the side of the vessel to loosen the remaining cells. Due to their high amount of adhesion proteins, epithelial cells tend to be very sticky on cell culture plastics. Do not over-trypsinize the cells, and gently wash the cells from the plastic.



3

Neutralize the trypsin and harvest the cells

Add 100 µl Trypsin Neutralization Solution per 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 300 x g.

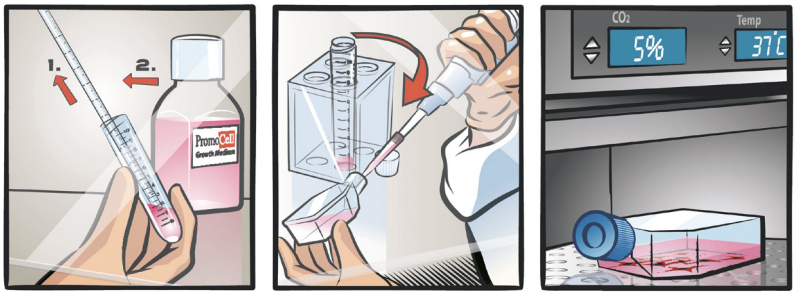


4

Incubate the cells

Discard the supernatant (step 1), add 1 ml of the appropriate PromoCell Cell Growth Medium, 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 prewarmed PromoCell Growth Medium. Place the vessels in an incubator (37°C, 5% CO_2) and change the media every two or three days.

Note: When culturing airway epithelial cells in Airway Epithelial Cell Growth Medium 2 or Airway Epithelial Cell Growth Medium XF (prf), the first medium change should not be performed before 24 hours.



Specifications

Product	Recommended Culture Media*	Plating Density	Passage after Thawing	Marker	Population Doublings
Human Nasal Epithelial Cells (HNEpC)	C-21040 or C-21050 or C-21060	5,000 – 15,000 cells per cm^2	P2	Cytokeratin$^+$	> 15
Human Tracheal Epithelial Cells (HTEpC)	C-21040 or C-21050 or C-21060	5,000 – 15,000 cells per cm^2	P2	Cytokeratin$^+$	> 15
Human Bronchial Epithelial Cells (HBEpC)	C-21040 or C-21050 or C-21060	5,000 – 15,000 cells per cm^2	P2	Cytokeratin$^+$	> 15
Human Small Airway Epithelial Cells (HSAEpC)	C-21070	5,000 – 15,000 cells per cm^2	P2	Cytokeratin$^+$	> 15

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

Related products

Product	Size	Catalog Number
Airway Epithelial Cell Growth Medium 2	500 ml	C-21040
Airway Epithelial Cell Growth Medium XF (prf)	500 ml	C-21050
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
Air-Liquid Interface Medium (ALI-Airway)	500 ml	C-21080
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