CI-pNAEC

Cat. No.: INS-CI-1029

Biosafety Level	Storage	Culture Conditions
Level 1	vapor phase of liquid nitrogen	Epithelial Cell Coating Solution (INS-SU-1020) huNASA Medium (INS-ME-1028)

General Information

CI-pNAEC is a porcine Nasal Epithelial Cell line that was immortalized using the CI-SCREEN technology (Lipps et al. 2018; *Nat Comm*). When cultivated on transwell, pNAEC cells form a barrier (TEER ~1000 Ω/cm^2), develop cilia, produce mucus and express tight-junction protein (ZO1).

Organism: Sus scrofa (pig)
Tissue: nasal epithelium
Growth properties: adherent

Cell culture media and reagents

Product	Cat. No.	Volume
huNASA Medium* (includes basal medium and supplements)	INS-ME-1028	500ml
Epithelial Cell Coating Solution	INS-SU-1020-20ml INS-SU-1020-100ml	20ml 100ml
Freezing medium	INS-SU-1004	30ml

***Note:** The medium does not contain antibiotics. However, it may simply be supplemented with standard antibiotics.*

Intended Use

This product is intended for in vitro research use only. It is not intended for any animal or human therapeutic or diagnostic use.

Quality control

Each vial contains ≥5×10^5 cells. Viability is ≥80%. Cells are negative for mycoplasma contamination. The source material is tested negative for HIV, HBV and HCV.

Upon arrival

Cells are routinely shipped on dry ice. Check all containers for leakage and breakage. Check if cells arrived frozen. After arrival, store the cryopreserved cells in liquid nitrogen vapor, or seed them immediately (please see page 2).

***Note:** Cells may be stored at -80°C for short periods (<2 days), but this results in reduced viability and irreversible cell damage.*

Coating of cell culture vessels

Material:

- Cell culture vessel(s)
- PBS
- Epithelial Cell Coating Solution (INS-SU-1020)

Protocol:

1) Cover the cell culture vessel with coating solution (see table below for the required volume).
2) Incubate the cell culture vessel for at least 2h (up to overnight) at 37°C in the incubator.
3) Aspirate coating solution.
4) Wash once with PBS and aspirate PBS.

***Note:** Coated cell culture vessels may be stored sealed at 2-8°C for up to 7 days.*

Vessel	Surface area (cm^2)	Volume (ml)
T75	75	2.5
T25	25	1.4
6well	9.6	0.7
12well	3.5	0.25
24 well	1.9	0.1
96well	0.32	0.05

Medium storage and preparation

Medium including supplements is shipped cooled at 4-8°C. Store reagents according to the instructions below upon arrival.

Material:

- huNASA Medium INS-ME-1028. Includes Basal Medium (500ml) and Supplements (30ml; INS-ME-1028BS).

Storage:

- Store Basal Medium at 4-8°C
- Store Supplements at -20°C
- Store completed medium (Basal Medium plus Supplements) at 4-8°C. Completed medium is stable for at least 1 month at 4-8°C.

Protocol:

1) Thaw Supplements at 15-25°C.
2) Add 30ml Supplements to 500ml Basal Medium and store at 4-8°C. Completed medium is stable for at least 1 month at 4-8°C.

Note: *Supplements may be aliquoted and stored at -20°C before completing the medium. For example, aliquot 5×6ml and then add 6ml Supplements to 100ml Basal Medium.*

Recover cryopreserved cells

Do not thaw the cells until the recommended medium and coated flasks are on hand. For initial recovery (after delivery), we recommend thawing the cells on a T25 flask.

Material:

- Coated cell culture vessels
- pre-warmed (37°C) complete medium (Basal Medium plus Supplements)
- 15ml tube

Protocol:

1) Add 4ml pre-warmed medium to a 15ml tube.
2) Quickly thaw the cryovial at 37°C in a water bath until only a few ice crystals are visible. Disinfect vial briefly with 70% Ethanol.
3) Transfer thawed cell suspension to the 15ml tube containing 4ml medium. Avoid excessive pipetting up and down.
4) Centrifuge cells at 200×*g* for 4min.
5) Aspirate supernatant.
6) Gently resuspend the cell pellet in complete medium.
7) Transfer cells in coated cell culture vessel and place in the incubator (37°C, 5% CO_2).
8) Change the medium after 2 days.

Routine Subculture

Change medium every 2 days and split the cells at 70-90% confluence. The split ratio after recovery from cryopreservation should not exceed 1:2. For routine maintenance, split ratio can be increased to 1:3 to 1:5.

Material:

- Coated cell culture vessels
- pre-warmed (37°C) complete medium (Basal Medium plus Supplements)
- PBS
- Trypsin/EDTA solution (TE)

Protocol:

1) Aspirate medium.
2) Wash with PBS and aspirate PBS.
3) Add Trypsin/EDTA (TE) solution to the cells and incubate at room temperature or 37°C for 5-10min, or until the cells detach.
4) Examine the cells under a microscope. When the cells start to detach, gently tap the side of the vessel to loosen the remaining cells.
5) Resuspend cells in complete medium thereby inactivating the Trypsin/EDTA (TE) solution.
6) Transfer an aliquot of the cell suspension to a new coated cell culture vessel containing fresh complete medium.
7) Incubate at 37°C and 5% CO_2.

Vessel	Medium or PBS (ml)	TE (ml)
T75 flask	8-10	2

T25 flask	4-5	1
6well	1.5-2	0.5
12well	1	0.2
24 well	0.5	0.1
96well	0.1	0.05

Cryopreservation

Cells should be grown to 90% confluence before cryopreservation. Avoid full confluence before cryopreservation.

Material:

- Freezing medium (INS-SU-1004)
- PBS
- Trypsin/EDTA solution (TE)
- 2% FBS in PBS
- 15ml tube
- cryovial(s)
- freezing container (“Mr. Frosty” or similar)

Protocol:

1) Aspirate medium.
2) Wash with PBS and aspirate PBS.
3) Add Trypsin/EDTA (TE) solution to the cells and incubate at room temperature or 37°C for 5-10min, or until the cells detach.
4) Examine the cells under a microscope. When the cells start to detach, gently tap the side of the vessel to loosen the remaining cells.
5) Resuspend cells in 2% FBS in PBS and transfer to a 15ml tube.
6) Centrifuge cells at 200×*g* for 4min.
7) Aspirate supernatant and gently resuspend cell pellet in Freezing medium (approx. 1×10^6 cells/ml).
8) Transfer cell suspension into cryovial(s) and place them into a freezing container (“Mr. Frosty” or similar).
9) Place the freezing container at -70 to -80°C for 16-24h.
10) Transfer cryovials to liquid nitrogen vapor for long-term-storage.

Cell Culture on Permeable Inserts

Cells can be cultivated on cell culture inserts at the Liquid-Liquid interface (LLI) or Air-Liquid interface (ALI). After approx. five weeks of ALI-culture, pNAECs show a differentiated phenotype.

Material:

- Epithelial Cell Coating Solution (INS-SU-1020) coated cell culture inserts (e.g., SABEU cellQART®, Corning Transwell® or similar products)
- standard multiwell plate (to hold the cell culture inserts)
- ⚠ pre-warmed (37°C) complete huNASA Medium (INS-ME-1028) for ALI culture
- PBS

Protocol:

1) Expand cells in routine monolayer culture using huNASA Medium.
2) Prepare coated cell culture inserts by adding the appropriate amount of coating solution to the required number of inserts.

Insert Type	Coating Volume (mL)	Wash Volume (mL)
6 well	0.6	1
12 well	0.25	0.4
24 well	0.05	0.1
96 well	0.025	0.05

3) Incubate the cell culture inserts for at least 2h (up to overnight) at 37°C in the incubator.
4) Aspirate coating solution.
5) Rinse once with PBS and aspirate PBS.
   Note: *Coated cell culture inserts may be stored sealed at 2-8°C for up to 7 days.*
6) Add the appropriate volume of huNASA Medium to the basal chamber of the plate holding the insert.

Insert Type	Medium Volume Basal Chamber/Well (mL)	Medium Volume Apical Chamber/Insert (mL)
6 well	2.0-2.6	1.5
12 well	1.0-1.5	0.5
24 well	0.5-0.6	0.1-0.2

Note: *Inserts from different manufacturers require different medium volumes. Please refer to insert manufacturer's instructions for details.*

7) Harvest expanded cells into huNASA Medium according to routine subculture protocol.

8) Count cells and seed 3×10^5 cells/cm^2 onto pre-coated cell culture inserts, e.g., 1×10^5 cells onto one 24-well insert using huNASA Medium.
9) Change medium every 2 days. Always aspirate from the basolateral side first, then the apical side. When replacing the media, add in the opposite order (apical then basolateral). Cells can be kept in LLI or air-lifted into ALI conditions.

Air-lift procedure for ALI culture:

- *Perform Air-Lift after 2-4 days, but earliest when cells are confluent.*

1) Aspirate the medium from both the basal and apical chambers. Add huNASA Medium to the basal chamber only.
2) Change medium every 2 days leaving the apical chamber empty.

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