CI-hAELVi

Immortalized human alveolar epithelial cells

Product Sheet

→ At a glance		
BSL Level 1	**Coating** huAEC Coating (INS-SU-1018)	**Growth** Adherent
Storage <2d: -80°C >2d: liquid Nitrogen	**Medium** huAEC Medium (INS-ME-1013)	**Cat.-No.** INS-CI-1015

→ Contents

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→ Intended Use and Licensing

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

If you have purchased this cell line for **academic, non-profit research**, the use of this cell line is governed by the **in**screenex *Limited Research Use License (LRUL)*. Please refer to the LRUL for the full terms and conditions, and relevant use limitations. If you have purchased this cell line for **commercial, for-profit research**, the use of this cell line is governed by the respective license agreement. Please refer to the agreement for the full terms and conditions, and relevant use limitations. If you wish to use the cell line for commercial, for-profit purposes please contact **licensing@inscreenex.com**.

→ Background Information

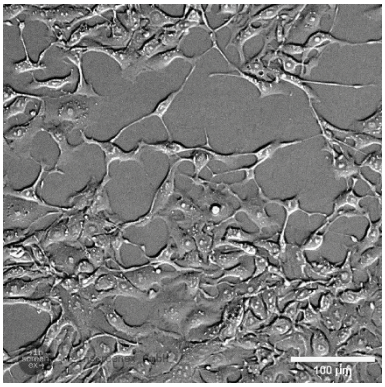
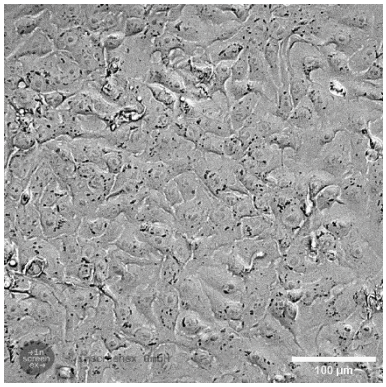
CI-hAELVi is an immortalized human alveolar epithelial cell line

Catalog number: *INS-CI-1015*
Biosafety level (BSL): *Level 1*
Organism: *Homo sapiens (human)*
Tissue: *lung*
Donor info: *male, Caucasian*
Growth properties: *adherent*

Studying the absorption and toxicity of inhaled drugs or chemicals requires a model reflecting the essential features of the air-blood barrier. For this purpose, InSCREENeX developed a novel alveolar type I cell line using the CI-SCREEN technology (recently published in Nature Communications; Lipps et al., 2018). In a joint collaboration project between the groups from Prof. Dr. Claus-Michael Lehr (Helmholtz Institute for Pharmaceutical Research Saarland (HIPS), Prof. Dr. Dagmar Wirth (Helmholtz Centre for Infection Research (HZI) and InSCREENeX the CI-hAELVi cell line was established and extensively characterized. This work is highlighted in the peer-reviewed paper published by Kuehn et al., 2016.

→ Morphology

epithelial-like; grows in monolayer;

Low confluency	***High confluency***
	

→ Cell Characterization and Assays

→ Marker Expression

The following cell-type specific markers were detected in CI-hAELVi:

Caveolin-1, occludin, ZO-1, pan-Cytokeratin, Cytokeratin18

→ Established Assays

CI-hAELVi were used in the following assays:

Air-liquid-Interface culture

Barrier formation (to model air-blood-barrier)

Infection studies

→ Quality Control

Basic information on quality control can be found below. For more details, request a Certificate of Analysis (CoA) by emailing **info@inscreenex.com** *and stating your Lot number.*

Cell number: *>0.5Mio viable cells*
Viability: *>75% post-thaw viability*
Sterility: *no contamination detected*
Mycoplasma: *no contamination detected*
Human pathogens: *negative for HBV, HCV, HIV-1/HIV-2*
STR: *Not available*

→ Related Products

Products that are related to the CI-hAELVi cell line and are either required for a successful cell culture or recommended.

Required	***Recommended***
Medium: *huAEC Medium (INS-ME-1013)* ***Coating solution:*** *huAEC Coating (INS-SU-1018)*	***Cryopreservation:*** *Freezing Medium (INS-SU-1004)* ***Related cell lines:*** *CI-huArlo (INS-CI-1031), CI-huBroBEC (INS-CI-1025)*

→ Upon Arrival

Cells are routinely shipped on dry ice. Check all containers for leakage and breakage. Check if cells arrived frozen.

If, immediately upon arrival:

- *the vial appears damaged,*
- *the dry ice level in the shipping container appears low,*
- *the cells appear thawed, or*
- *you have any other concerns regarding the quality of the cells,*

do the following:

1) *take photos of the vial and/or the shipping container,*
2) *contact us by email or telephone (see* **General Inquiries** *on page 2).*

If everything looks good, either seed the cryopreserved cells immediately, or store them:

- *at -80°C for periods of up to 2 days, or*
- *below -130°C in liquid nitrogen vapor, for long term storage.*

→ Coating Protocol

Our *huAEC Coating is a sterile, ready-to-use solution ideal for coating cell culture surfaces.*

<table>
<tr><th>Required materials</th><th>Protocol</th></tr>
<tr><td>

- *Cell culture plastic (flasks, dishes, plates*
- *Sterile PBS*
- *huAEC Coating (INS-SU-1018)*

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1) *Cover the cell culture vessel with huAEC Coating (see table to the left for recommended volumes). Rock back and forth to cover entire surface if necessary.*
2) *Incubate for at least 2h at 37°C in the incubator or overnight at 2-8°C.*
3) *Aspirate huAEC Coating.*

</td></tr>
<tr><td>Storage</td></tr>
<tr><td>

- *Store huAEC Coating at 2-8°C, if not indicated otherwise on the product label.*

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- *Store coated cell culture plastic sealed at 2–8°C for up to 7 days.*

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4) *Wash once with at least 4 volumes of sterile PBS.*
5) *Aspirate PBS.*
6) *Use immediately or store coated cell culture plastic according to instructions provided to the left.*

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Recommended volumes

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Flask/Plate	***Coating Volume***
T75	*3ml*
T25	*1ml*
6-well	*0.7ml*
12-well	*0.25ml*
24-well	*0.1ml*
48-well	*75µl*
96-well	*50µl*

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→ Medium Storage and Preparation

Our huAEC Medium is shipped as a kit that consists of:

- *30ml Supplements*
- *500ml Basal Medium*

Both components need to be combined according to the instructions below to obtain the complete, ready-to-use medium.

→ Cell culture antibiotics: *The medium does not contain prophylactic antibiotics. If you wish to use antibiotics, any standard, cell culture grade antibiotics can be added to the medium.*

Required materials	***Protocol***
- *30ml Supplements* - *500ml Basal Medium*	1) *Thaw Supplements at ambient temperature.*
Storage	

– *Store* **Supplements** *at -20°C, if not indicated otherwise on the product label.* – *Store* **Basal Medium** *at 2-8°C, if not indicated otherwise on the product label.* – *Store* **complete Medium** *at 2-8°C. Complete Medium is stable for at least 1 month at 2-8°C.*	*2) Add 30ml Supplements to 500ml Basal Medium to obtain the complete Medium.* *To prepare smaller volumes of complete Medium, aliquot Supplements and store at -20°C. For example, aliquot 5×6ml and then add 6ml Supplements to 100ml Basal Medium.*

→ Thaw Cryopreserved Cells

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

Required materials	***Protocol***
– *Coated cell culture vessel (see section* → **Coating** Protocol *on page 5)* – *Complete Medium, pre-warmed to 37°C (see section* → **Medium Storage and** Preparation *on page 6)* – *15ml tube with a conical bottom suitable for centrifugation (e.g. "Falcon tube")*	*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 by spraying with 70% Ethanol.* *3) Transfer thawed cell suspension to the 15ml tube containing 4ml medium. Avoid excessively pipetting up and down.* *4) Centrifuge cells at 300×g for 5min.* *5) Aspirate supernatant.* *6) Gently resuspend the cell pellet in complete Medium. Use a volume appropriate for the cell culture vessel.*

	7) Transfer cells in coated cell culture vessel and place in the incubator (37°C, 5% CO2). 8) Change the medium after 2 days.

→ Freeze Cells for Cryopreservation

Cell should be grown to 80-90% confluence before cryopreservation. Avoid full confluence before cryopreservation.

Required materials	**Protocol**
– Freezing medium (INS-SU-1004) – PBS – Trypsin/EDTA solution (TE) – 2% FBS in PBS – 15ml tube – Cryovial(s) – Freezing container (“Mr. Frosty” or similar) – 15ml tube with a conical bottom suitable for centrifugation (e.g. "Falcon tube")	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 conical bottom tube. 6) Centrifuge cells at 300×g for 5min. 7) Aspirate supernatant and gently resuspend cell pellet in Freezing medium (approx. 1Mio. 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-80°C for 16-24h. 10) Transfer cryovials to liquid nitrogen vapor for long-term-storage. Add 4ml pre-warmed medium to a 15ml tube.

→ Routine Cell Culture

Work in a sterile environment and follow Good Cell and Tissue Culture Practice.

Temperature: *37°C*
Environment: *5% CO_2 (v/v), humidified atmosphere*
Split ratio: *1:2 for initial split after thawing, 1:5 to 1:10 for routine culture*
Confluence: *split at 70-90% confluence*
Medium change: *every 2-3 days*

Required materials	***Protocol***
– Coated cell culture vessel (see section → **Coating** Protocol on page 5) – Complete Medium, pre-warmed to 37°C (see section → **Medium Storage and** Preparation on page 6) – PBS – Trypsin/EDTA solution (TE)	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.

Recommended volumes	
<table><tr><th>Flask or Plate</th><th>Medium or PBS</th><th>TE solution</th></tr><tr><td>T75</td><td>8–10ml</td><td>3ml</td></tr><tr><td>T25</td><td>4–5ml</td><td>1ml</td></tr><tr><td>6-well</td><td>1.5–3ml</td><td>0.7ml</td></tr><tr><td>12-well</td><td>1–2ml</td><td>0.25ml</td></tr><tr><td>24-well</td><td>0.5–1ml</td><td>0.1ml</td></tr><tr><td>48-well</td><td>0.2–0.4ml</td><td>75µl</td></tr><tr><td>96-well</td><td>0.1–0.2ml</td><td>50µl</td></tr></table>	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) Place into incubator.

→ Culture on Inserts

Cells can be cultivated on cell culture inserts at the Liquid-Liquid interface (LLI) and Air-Liquid interface (ALI).

Note: The specifications provided in this manual pertain to the products used specified (culture vessels, inserts). Variations may occur with other products.

→ Liquid-Liquid Interface (LLI)

Required materials	**Protocol**
– Coated cell culture inserts (e.g., SABEU cellQART®, Corning Transwell® or similar products), (see section → **Coating** Protocol on page 5) – compatible multiwell plate (to hold the cell culture inserts) – Complete Medium, pre-warmed to 37°C (see section → **Medium Storage and** Preparation on page 6) – PBS – Trypsin/EDTA solution (TE)	1) Expand cells in routine monolayer culture using complete Medium. 2) Prepare coated cell culture inserts by adding the appropriate amount of coating solution to the required number of inserts. 3) Incubate the cell culture inserts for at least 2h (up to overnight) at 37°C in the incubator. 4) Aspirate coating solution.

Recommended volumes for insert coating

Insert Type	Coating Volume	Wash Volume
6 well	0.6ml	1.0ml
12 well	0.25ml	0.4ml
24 well	0.05ml	0.1ml
96 well	0.025ml	0.05ml

Recommended Medium volumes for insert cultivation

Insert Type	Baso-lateral	Apical
6 well	2.0-2.6ml	1.5ml
12 well	1.0-1.5ml	0.5ml
24 well	0.5-0.6ml	0.1-0.2ml

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) Harvest expanded cells into Complete Medium according to routine subculture protocol.
7) Count cells and seed 3×10^5 cells/cm^2 onto precoated cell culture inserts, e.g., 1×10^5 cells onto one 24-well insert using Complete Medium.
8) Add the appropriate volume of Complete Medium to the basal chamber of the plate holding the insert.
9) Change medium every 2 days. Always aspirate from the basolateral (below) side first, then the apical side (obove). 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-Liquid Interface (ALI)

Air-lift procedure for ALI culture:

Perform Air-Lift after 2-4 days of Liquid-Liquid-culture (see protocol above), but earliest when cells are confluent.

1) Aspirate the medium from both the basal and apical chambers. Add Complete Medium to the basal chamber only.

2) Change medium every 2 days, leave the apical chamber empty.

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