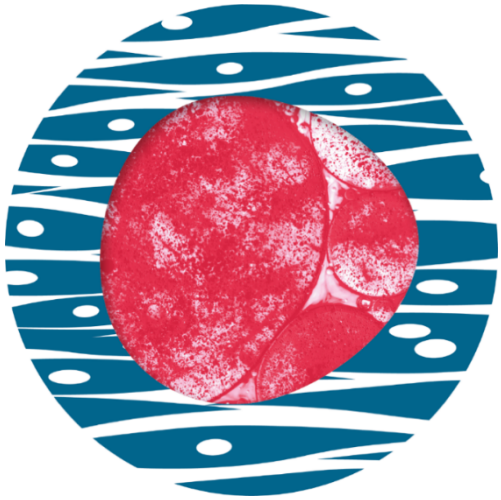
CI-huVEC

Immortalized human umbilical vein endothelial cells

Product Sheet

→ At a glance		
BSL Level 1	**Coating** Coating Solution (INS-SU-1026)	**Growth** Adherent
Storage <2d: -80°C >2d: liquid Nitrogen	**Medium** huVEC Medium (INS-ME-1011)	**Cat.-No.** INS-CI-1002



→ Contents

→ Intended Use and Licensing 2

→ Background Information 3

→ Morphology 3

→ Cell Characterization and Assays 3

  → Marker Expression 3

  → Established Assays 4

→ Quality Control 4

→ Related Products 4

→ Upon Arrival 5

→ Coating Protocol 5

→ Medium Storage and Preparation 6

→ Thaw Cryopreserved Cells 7

→ Freeze Cells for Cryopreservation 8

→ Routine Cell Culture 9

→ References 10

inscreenex **GmbH**
Inhoffenstr. 7
38124 Braunschweig
Germany

→ General Inquiries
M. info@inscreenex.com
T. +49 531 6181 5080

→ Ordering
M. orders@inscreenex.com

→ Licensing
M. licensing@inscreenex.com

→ 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

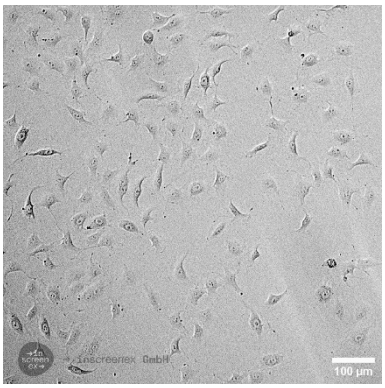
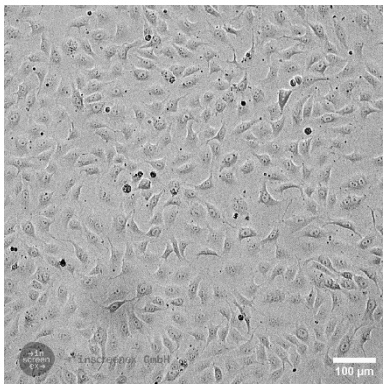
CI-huVEC is an immortalized Immortalized human umbilical vein endothelial cell line.

Catalog number: *INS-CI-1002*
Biosafety level (BSL): *Level 1*
Organism: *Homo Sapiens (human)*
Tissue: *Umbilical cord*
Donor info: *female*
Growth properties: *adherent*

CI-huVECs are immortalized human umbilical vein endothelial cells (HUVECs). HUVECs line the endothelium of the umbilical cord vein, where they play a key role in maintaining vascular function, homeostasis, and various vascular processes. HUVECs in vitro are widely used in research as a model for studying endothelial cell biology and various physiological processes including macromolecule transport, blood coagulation, angiogenesis, and fibrinolysis.

→ Morphology

adherent; growth in monolayer; cobblestone appearance

Low confluency	***High confluency***
	

→ Cell Characterization and Assays

→ Marker Expression

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

CD31, CD309 (VEGFR-2), von Willebrand factor (vWF)

→ Established Assays

CI-huVEC were used in the following assays:

Not available

→ 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: *primary material negative for HBV, HCV, HIV-1/HIV-2*
STR: *TBD*

→ Related Products

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

Required	***Recommended***
Medium: *huVEC Medium (INS-ME-1011)* ***Coating solution:*** *Coating Solution (INS-SU-1026)*	***Cryopreservation:*** *Cell Freezing Medium (INS-SU-1027)* ***Related cell lines:*** *CI-huMEC (INS-CI-1008)*

→ 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 Coating Solution is a ready-to-use solutions, ideal for coating cell culture surfaces.

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

- *Cell culture plastic (flasks, dishes, plates*
- *Coating Solution (INS-SU-1026)*

</td><td rowspan="3">

1) *Cover the cell culture vessel with Coating Solution (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 Coating Solution.*

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

- *Store Coating Solution at 2-8°C, if not indicated otherwise on the product label.*
- *Store coated cell culture plastic sealed at 2-8°C for up to 7 days.*

</td></tr>
</table>

Recommended volumes	4) Use immediately or store coated cell culture plastic according to instructions provided to the left.

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

→ Medium Storage and Preparation

Our huVEC 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. 2) Add 30ml Supplements to 500ml Basal Medium to obtain the complete Medium.
Storage	
– Store **Supplements** at -20°C, if not indicated otherwise on the product label.	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.

– *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.*	

→ 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***
– *Cell Freezing Medium (INS-SU-1027)* – *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 Cell 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.* *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.*
Recommended volumes	

Flask or Plate	***Medium or PBS***	***TE solution***
T75	*8-10ml*	*3ml*
T25	*4-5ml*	*1ml*
6-well	*1.5-3ml*	*0.7ml*
12-well	*1-2ml*	*0.25ml*
24-well	*0.5-1ml*	*0.1ml*
48-well	*0.2-0.4ml*	*75µl*
96-well	*0.1-0.2ml*	*50µl*

→ References

Heiss, Maximilian et al. “Endothelial cell spheroids as a versatile tool to study angiogenesis in vitro.” FASEB journal vol. 29,7 (2015): 3076-84. doi:10.1096/fj.14-267633

Lipps, Christoph et al. “Expansion of functional personalized cells with specific transgene combinations.” Nature communications vol. 9,1 994. 8 Mar. 2018, doi:10.1038/s41467-018-03408-4

Schwefel, Konrad et al. “Biallelic CCM3 mutations cause a clonogenic survival advantage and endothelial cell stiffening.” Journal of cellular and molecular medicine vol. 23,3 (2019): 1771-1783. doi:10.1111/jcmm.14075

Schwefel, Konrad, et al. "Fibronectin rescues aberrant phenotype of endothelial cells lacking either CCM1, CCM2 or CCM3." The FASEB Journal 34.7 (2020): 9018-9033.

Much, Christiane D et al. “Inactivation of Cerebral Cavernous Malformation Genes Results in Accumulation of von Willebrand Factor and Redistribution of Weibel-Palade Bodies in Endothelial Cells.” Frontiers in molecular biosciences vol. 8 622547. 9 Jul. 2021, doi:10.3389/fmolb.2021.622547

Rath, Matthias, et al. "Contact-dependent signaling triggers tumor-like proliferation of CCM3 knockout endothelial cells in co-culture with wild-type cells." Cellular and Molecular Life Sciences 79.6 (2022): 340.

Dienemann, Sandra et al. “Comparative analysis of hypoxic response of human microvascular and umbilical vein endothelial cells in 2D and 3D cell culture systems.” Journal of cellular physiology vol. 238,5 (2023): 1111-1120. doi:10.1002/jcp.31002

Trossmann, Vanessa Tanja, and Thomas Scheibel. "Design of Recombinant Spider Silk Proteins for Cell Type Specific Binding." Advanced Healthcare Materials 12.9 (2023): 2202660.