

Protocol – Version 1.0

Human Umbilical Vein Endothelial Cells

Catalog No.	Product Name	Product quantity	Short-term Storage	Long-term Storage	Thawing Instructions
ax3811	Umbilical Vein Endothelial Cells	500,000 cells/vial	Liquid nitrogen	Liquid nitrogen	See below
ax3812	Umbilical Vein Endothelial Cell Culture Medium	500 mL	4°C for 1 month	-20°C for 6 months	Thaw at 4°C or RT

Lot-specific information such as donor details and passage number are stated in the Certificate of Analysis for each product.

Recommendations:

Always count the number of viable cells after thawing

Recommended culture vessel coating: Not required

• Recommended cell culture medium: Umbilical Vein Endothelial Cell

Culture Medium

Recommended seeding density: 2,500-5,000 viable cells/cm²

Recommended centrifugation speed: 150 x g for 5 min

Thawing & Plating:

- Transfer the vial of cells from liquid nitrogen storage with the vial buried in dry ice. Remove the vial from dry ice and transfer it immediately to a 37°C water bath.
- Thaw the cells quickly in a 37°C water bath until just prior to complete thawing.
- Wipe the outside of the vial with 70% ethanol.
- Gently resuspend the cells and take an aliquot to perform a cell count.
- Immediately after thawing, slowly dilute the cells into the required volume of pre-warmed Umbilical Vein Endothelial Cell Culture Medium (must be at least 10 mL so that the concentration of DMSO is less than 1%).
- Rinse the cryovial with 1 mL of Umbilical Vein Endothelial Cell Culture Medium to ensure all of the cells are transferred.
- Seed cells into the culture vessel at the recommended seeding density of 2,500-5,000 viable cells/cm².
- Incubate the cells at 37°C, 5% CO₂ in a humidified incubator.
- Once the cells have attached (after 6-24 h), replace the culture medium with fresh, prewarmed Umbilical Vein Endothelial Cell Culture Medium.
- Frequency of media changes: Every 2 days

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Passaging:

The endothelial cells should not be allowed to reach 100% confluency since the growth rate may reduce. For maximal growth, passage cells before confluency.

Passage when the culture reaches: 80% confluent
 Recommended passaging reagent: Trypsin-EDTA

- After adding passaging reagent, incubate the cells for 5 min at 37°C. Observe the cells at regular intervals for detachment from the culture vessel.
- Once the cells have detached, dilute out the trypsin with pre-warmed Umbilical Vein Endothelial Cell Culture Medium.
- Centrifuge the cells at 150 x g for 5 min.
- Remove the supernatant and resuspend the cell pellet in 1-2 mL of pre-warmed Umbilical
 Vein Endothelial Cell Culture Medium.
- Perform a cell count to determine the number of viable cells.
- Dilute the cells into the required volume of pre-warmed Umbilical Vein Endothelial Cell Culture Medium.
- Seed cells into the culture vessel at the recommended seeding density of 2,500-5,000 viable cells/cm².
- Incubate the cells at 37°C, 5% CO₂ in a humidified incubator.

Usage Statement:

Our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, *in vitro* diagnostic uses, *ex vivo* or *in vivo* therapeutic uses or any type of consumption or application to humans.

Got any questions? Need help with the protocol?

Contact Axol Technical Support at support@axolbio.com
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