



## Human Umbilical Vein Endothelial Cells (Primary)

**Catalog Number:** NBP-0001  
**Product Format:** Frozen  
**Vial Cell Number:** 500,000cells  
**Passage:**1(P1)  
**Storage:** Liquid Nitrogen, Vapor Phase  
**Intended Use:** Research use only (RUO)

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### General Information:

HUVECs (Human Umbilical Vein Endothelial Cells; catalogue number NBP-0001) are expertly isolated from pooled, healthy human umbilical veins, making them a crucial cellular model for investigating endothelial function and related diseases. These cells serve as a cornerstone in vascular biology research due to their pivotal role in a variety of physiological and pathological processes, including angiogenesis, inflammation, and blood vessel function.

Upon shipping, HUVECs are carefully provided in frozen vials at passage 1. This early passage is specifically chosen to ensure the maximum vitality and performance of the cells when cultured, thereby supporting optimum results in experimental applications. To facilitate proper growth and maintenance of these cells, it is recommended to use Endothelial Growth Medium (EGM; catalogue number NBP-02), which is rich in nutrients, containing 10% serum along with essential growth supplements. Under the recommended culture conditions, HUVECs can achieve a minimum average population doubling level exceeding 20, providing a reliable and reproducible cell source for experimental use.

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### CULTURE REQUIREMENTS

<b>Component</b>	<b>Recommendation</b>
<b>Basal Medium</b>	Endothelial Basal Medium (EBM)
<b>Growth Supplement</b>	Endothelial Growth Supplement
<b>Serum</b>	10% Fetal Bovine Serum (FBS)
<b>Coating Matrix</b>	<b>Required:</b> NeoCoating Solution
<b>Culture Vessel</b>	Pre-coated tissue culture plastic
<b>Incubation</b>	37°C, 5% CO <sub>2</sub> , 95% humidity
<b>Split Ratio</b>	1:2 to 1:4 every 3-5 days
<b>Maximum Passages</b>	Recommended: P2-P6



## CELL CHARACTERISTICS

Property	Description
<b>Morphology</b>	Cobblestone monolayer at confluence
<b>Marker Expression</b>	CD31/PECAM-1+, vWF/Factor VIII+, VE-cadherin+, CD34+ (variable), Uptake of Dil-Ac-LDL
<b>Growth Pattern</b>	Contact-inhibited, non-transformed
<b>Typical Doubling Time</b>	24-48 hours
<b>Angiogenesis Capacity</b>	Tube formation in Matrigel® assay
<b>Applications</b>	Vascular biology, angiogenesis assays, inflammation studies, drug screening, permeability studies

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### Product Usage:

Please note that these cells are strictly intended for research purposes only. All users must comply with relevant guidelines and laboratory protocols to ensure proper handling and usage, contributing to a safe research environment.

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### Shipping:

The frozen vials are shipped securely within a dry ice package. This critical shipping method maintains the integrity of the cells during transit, ensuring that they arrive in peak condition for immediate use or for storage and future experiments.

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### Handling Instructions upon Arrival:

Upon receipt of the dry ice package containing the frozen vials, it is essential to act swiftly. Transfer the vials directly into a -80°C freezer for short-term storage, or alternatively, into a liquid nitrogen tank for long-term preservation. This immediate action is vital to maintain cell viability and functionality, ensuring optimal performance when the cells are ultimately utilized.



## **Protocols for Thawing and Subculture:**

### **A) Pre-coating of T25 Flasks:**

Before seeding the HUVECs, prepare the T25 flasks by adding 2 ml of Cell Coating Solution (NBP-01) to guarantee complete coverage of the flask surface. Allow the coating to settle for a period of 5 minutes, then aspirate any excess solution to prepare the flask for optimal cell attachment. While alternative coating solutions, such as gelatin, collagen, or fibronectin, can be used, it's advisable to optimize the specific conditions for each alternative prior to initial use to ensure effective cell attachment.

### **B) Thawing the Cells:**

To successfully revive the frozen HUVECs, immerse the vial in a water bath set at 37°C until completely thawed. Once thawing is complete, transfer the cells carefully into the pre-coated T25 flask, adding 10 ml of NBP-02 medium. Under ideal culture conditions, these cells are expected to reach confluence within 24 hours, making them available for passage by the next day.

### **C) Passaging the Cells:**

When the cells attain approximately 80-90% confluence, initiate the passaging process. Begin by rinsing the cells in the T25 flask twice with 5 ml of Hank's Balanced Salt Solution (HBSS) at room temperature to remove any residual media. To detach the cells from the flask surface, add 2 ml of Trypsin/EDTA (NBP-23). It is critical to gently aspirate the excess Trypsin/EDTA solution within 20 seconds to prevent prolonged exposure, which could adversely affect cell viability.

### **D) Cell Detachment:**

After adding Trypsin/EDTA, allow the T25 flask to sit at room temperature or within a 37°C incubator for 1 minute. Most cells typically begin detaching within 1-2 minutes. It is recommended to monitor this detachment process under a microscope; when a majority of cells appear rounded, gently tap the flask against the bench surface to encourage the movement of any unattached cells.

### **E) Cell Collection:**

Once detachment is confirmed, add 5 ml of Trypsin Neutralization Buffer (NBP-024) to halt the enzymatic activity. Following this, centrifuge the resulting cell suspension at 800 RPM for 5 minutes to pellet the cells at the bottom of the tube.

### **F) Resuspension and Transferring:**

Carefully resuspend the cell pellet in either 10 or 15 ml of NBP-02 medium, based on the desired cell density. For effective subculture, aliquot 5 ml into two or three pre-coated T25 flasks to maintain a subculture ratio of 1:2 to 1:3.



### G) Medium Change:

To promote optimal growth and health of the HUVECs, change the culture medium every 2 to 3 days. Adhering to a subculture ratio of 1:3 generally allows the cells to achieve confluence within a week, ensuring a consistent supply of cells for experimental needs.

### H) Preparing Quiescent Cells:

When preparing cells for specific experimental procedures, replace the NBP-02 medium with Endothelial Basal Medium (EBM; catalogue number NBP-03) containing 0.5% FBS once the cells are near confluence. Incubate in this medium for approximately 8-12 hours to induce a quiescent state, thus creating a stable environment that is conducive to various experimental setups.

This comprehensive protocol ensures optimal culture conditions and prepares HUVECs for a wide range of research applications focused on endothelial biology.

#### Related products

NeoCoating Solution	NBP-01	240ml	NeoBioPharma
Endothelial Growth Medium	NBP-02	500ml	NeoBioPharma
Endothelial Basal Medium	NBP-03	500ml	NeoBioPharma
HBSS w/o Ca <sup>2+</sup> , Mg <sup>2+</sup>	NBP-11	100ml	NeoBioPharma
Cell Freezing Solution (FBS)	NBP-22	50ml	NeoBioPharma
Cell Freezing Solution (Non-FBS)	NBP-22B	50ml	NeoBioPharma
Trypsin/EDTA Solution	NBP-23	100ml	NeoBioPharma
Trypsin Neutralization Solution	NBP-28	100ml	NeoBioPharma
ITS (100x)	NBP-26	10ml	NeoBioPharma
L-Glutamine-MAXIMUM (100x)	NBP-27	100ml	NeoBioPharma
Human Plasma Fibronectin Solution	NBP-42	1mg/ml	NeoBioPharma

#### Caution:

Human tissue-derived products may contain biological hazards. Even though each cell strain is screened and found negative for major pathogens such as HIV, HBV, and HCV, as well as for detectable DNA contaminants, no diagnostic test is perfectly reliable. As a result, there is always a residual risk of exposure to infectious agents. To avoid contamination, always wear gloves and safety glasses when working with these materials. Never mouth pipette. These precautions represent the minimum level of care required to reduce the risk of contamination or exposure when working with human tissue-derived products