

# Primary Cells

## Human Umbilical Vein Endothelial Cells (HUVECs), Frozen



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Catalog #200-0630

5 x 10<sup>5</sup> cells

## Product Description

Human umbilical vein endothelial cells (HUVECs) were isolated from healthy single-donor umbilical cord veins using the collagenase digestion method. The cells were cultured and expanded with the EC-Cult™-XF Culture Kit (Catalog #08000) for two passages before they were cryopreserved in CryoStor® CS10 (Catalog #07930). Prior to cryopreservation, these HUVECs were ≥ 90% viable, and they are expected to have > 16 population doublings post-thaw. HUVECs can be used with the EC-Cult™-XF Culture Kit as an endothelial cell model to study physiological and pathological pathways, such as inflammation, angiogenesis, oxidative stress, hypoxia, and the formation of blood vessels.

Cells were obtained using Institutional Review Board (IRB)-approved consent forms and protocols.

Donor Status:	Normal
Characterization Criteria:	Cell count, viability, donor virus testing, sex (if available), other information
Format:	HUVECs are frozen in CryoStor® CS10.
Purity:	≥ 95% CD31+CD144+ and ≤ 5% CD45+ by flow cytometry
Expected Population Doublings:	16+

For donor details, refer to the lot-specific Certificate of Analysis.

## Stability and Storage

Product stable at -135°C or colder for 12 months from date of receipt. Short-term storage of cells (< 1 month) at -80°C is acceptable, but should be minimized to ensure maximum stability. Thawed samples must be used immediately. As these are primary cells, they have a finite life span in culture.

## Precautions

Donor Screening: Donors are screened for HIV-1 and -2, hepatitis B, and hepatitis C.

Cryopreserved products are shipped with negative test results from donor screening that is done within 90 days of collection.

Donors have been tested and found to be negative for HIV-1 and -2, hepatitis B, and hepatitis C prior to donation. As testing cannot completely guarantee that the donor was virus-free, THIS PRODUCT SHOULD BE TREATED AS POTENTIALLY INFECTIOUS and only used following appropriate handling precautions such as those described in biological safety level 2.

Storage of frozen cell products in the vapor phase of a liquid nitrogen storage tank is recommended. Storage in the liquid phase can result in cross contamination if the vial breaks or is not sealed properly. Storage in the liquid phase also increases the potential for liquid nitrogen to penetrate the vial and cause it to explode when removed from storage. Use of a face shield is required as a safety precaution when transferring cells from one container to another. When handling this product do not use sharps such as needles and syringes.

STEMCELL cannot guarantee the biological function or any other properties associated with performance of cells in a researcher's individual assay or culture systems. STEMCELL assures the cells will meet the specifications only when assessed immediately after thawing (before washing) by our test methods.

FOR IN VITRO RESEARCH USE ONLY. NOT APPROVED FOR DIAGNOSTIC, THERAPEUTIC, OR CLINICAL APPLICATIONS.

NOT APPROVED FOR HUMAN OR VETERINARY USE IN VIVO.

## Handling/Directions for Use

**IMPORTANT:** To confirm the number of cells provided, a viable cell count must be done immediately after thawing (before washing). Work quickly once the cells have been thawed to ensure high viability and recovery. Use sterile technique when processing thawed cells.

**NOTE:** When thawing HUVECs, use EC-Cult™-XF Culture Kit for all steps requiring the addition of medium (i.e. rinsing, washing, and resuspension).

1. Warm medium in a 37°C water bath.
2. Wipe the outside of the vial of cells with 70% ethanol or isopropanol.
3. In a biosafety hood, twist the cap a quarter-turn to relieve internal pressure and then retighten.
4. Quickly thaw cells in a 37°C water bath by gently shaking the vial. Remove the vial when a small frozen cell pellet remains. Do not vortex cells.

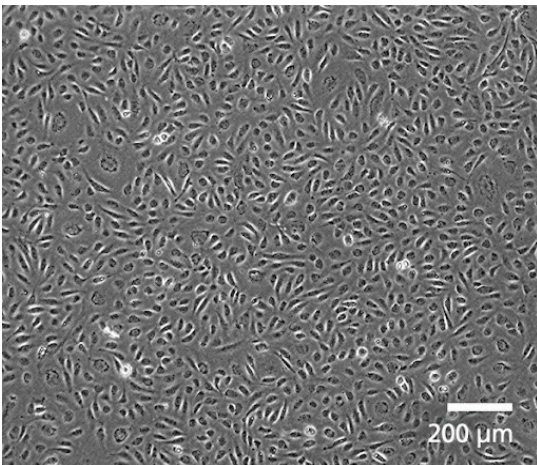
**NOTE:** It is important to work quickly in the following steps to ensure high cell viability and recovery.

5. Wipe the outside of the vial with 70% ethanol or isopropanol.
6. Measure the total volume of the cell suspension using a 2 mL serological pipette. This value is used in step 12 to calculate the number of cells provided.
7. Remove a 20 µL aliquot of cells for counting and set aliquot aside until step 12.
8. Transfer the remaining cell suspension to a 50 mL conical tube.
9. Rinse the vial with 1 mL of medium and add it dropwise to the cells, while gently swirling the 50 mL tube.
10. Wash by adding 15 - 20 mL of medium dropwise, while gently swirling the tube.
11. Centrifuge the cell suspension at 300 x g for 10 minutes at room temperature (15 - 25°C).
12. During centrifugation, dilute the aliquot from step 7 in 20 µL of Trypan Blue and perform a total nucleated cell count. See Tips section for more details on performing cell counts with a hemocytometer.
13. Carefully remove the supernatant with a pipette, leaving a small amount of medium to ensure the cell pellet is not disturbed. Resuspend the cell pellet by gently flicking the tube.

**NOTE:** Cell loss of up to 30% can be expected during the wash step.

14. Cells are now ready for use in downstream applications.

**NOTE:** Passage HUVECs at 90% confluency; refer to Figure 1 for a representative image. For complete instructions on culturing HUVECs using EC-CULT™-XF Culture Kit, refer to the applicable Product Information Sheet (Document #DX20709), available at [www.stemcell.com](http://www.stemcell.com), or contact us to request a copy.



**Figure 1. Phase Contrast Image of HUVECs Cultured in EC-Cult™-XF**

## Tips

For a protocol on performing total nucleated cell counts using a hemocytometer, refer to <https://www.stemcell.com/how-to-count-cells-with-a-hemocytometer>.

### Accessory Products

PRODUCT NAME	CATALOG #
EC-Cult™-XF Culture Kit	08000
Trypan Blue	07050
Falcon® Conical Tubes, 50 mL	38010
Falcon® Serological Pipettes, 2 mL	38002
CryoStor® CS10	07940
Animal Component-Free Cell Dissociation Kit	05426

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