Corning® HUVEC-2

Human Umbilical Vein Endothelial Cells

Cryopreserved Secondary Culture

Cat. No. 354151

≥ 500,000 viable cells/vial

Instructions for storage, initiation of cultures from cryopreserved cells, and subculture

Caution: Although cryopreserved cells from Corning Life Sciences have been tested for the presence of various hazardous agents, diagnostic tests are not necessarily 100% accurate. In addition, human cells may harbor other known or unknown agents or organisms, which could be harmful to your health or cause fatal illness. The user should treat all human cells as potential pathogens. *Use Universal Precautions*. Wear protective clothing and eyewear. Practice appropriate disposal techniques for potentially pathogenic or biohazardous materials.

Product Description

Corning Human Umbilical Vein Endothelial Cells (HUVEC-2) are derived from single donors and cryopreserved at the end of passage level 1 in a medium containing 10% DMSO. Each vial of this product contains ≥5 x 10^5 viable cells that have been tested to assure a robust migratory response to angiogenic factors such as VEGF and FBS. Single donor primary HUVEC-2 cells are suitable for use in combination with Corning BioCoat™ Angiogenesis Assay Systems to provide *in vitro* models for angiogenesis (e.g., cardiovascular, vascular, wound healing) and cancer research. For recommended precautions to be taken when handling human cells, please read the caution box above this product description.

Intended Use

Cryopreserved HUVEC-2 cells are prequalifed cells for use in Corning BioCoat Angiogenesis System: Endothelial Cell Migration. May also be used in Corning BioCoat Endothelial Cell Invasion and Tube Formation Assays.

Storage and Stability

Cryopreserved HUVEC-2 cells should arrive frozen on dry ice. If the cells are not to be used immediately, the user should prepare a space for storage of the vial in the vapor phase of a liquid nitrogen freezer. While wearing protective eyewear, gloves, and a laboratory coat, remove the vial from its shipping container and place immediately in the liquid nitrogen freezer. Although the viability of cryopreserved cells decreases with time in storage, useful cultures can usually be established even after two years of storage at liquid nitrogen temperatures.

Initiating Cultures from Cryopreserved Cells

We recommend seeding HUVEC-2 cells recovered from cryopreservation at a density of 2.5 x 10^3 viable cells/cm^2. For example, three 75 cm^2 or nine 25 cm^2 tissue culture flasks can usually be established from one vial containing ≥5 x 10^5 HUVEC-2 cells. The procedure given below is a sample protocol for establishing cultures from the contents of one vial.

1. Prepare a beaker of water at 37°C.
2. Remove a vial of HUVEC-2 cells from liquid nitrogen storage, taking care to protect hands and eyes.
3. Loosen the cap on the vial 1/4 turn for 10 seconds to release any liquid nitrogen that may be trapped in the threads, then re-tighten the cap.
4. Dip the lower half of the vial into the 37°C water to thaw.
5. When the contents of the vial have thawed, wipe the outside of the vial with disinfecting solution and move to a Class II, type A laminar flow culture hood.
6. Open the vial and pipette the suspension up and down with a 1 ml pipette to disperse the cells.
7. Remove 20 µl from the vial and dilute the cell suspension in 20 µl of trypan blue solution (for example: Sigma Chemical Company's Cat. No. T8154).
8. Using a hemacytometer, determine the number of viable cells/ml.
9. Use Cascade Biologics' Medium 200 (Cat. No. M-200-500) or Medium 200PRF (Cat. No. M-200PRF-500) supplemented with Cascade Biologics' LSGS (Cat. No. S-003-10) or LSGS Kit (Cat. No. S-003-K) to dilute the contents of the vial (1 ml) to a concentration of 1.25 x 10^4 viable cells/ml.
10. Add 5 ml of cell suspension to each 25 cm^2 culture flask or 15 ml of cell suspension to each 75 cm^2 culture flask.

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For Research Use Only. Not for use in diagnostic or therapeutic procedures.

11. Following inoculation, swirl the medium in the flasks to distribute the cells. HUVEC-2 cells attach to culture surfaces quickly, and if the medium is not distributed immediately following inoculation, the cells may grow in uneven patterns.
12. Incubate the cultures in a 37°C, 5% CO_2/95% air, humidified cell culture incubator. For best results, do not disturb the culture for at least 24 hours after the culture has been initiated.

Maintenance of Stock Cultures

1. Change the culture medium to fresh supplemented Medium 200 or Medium 200PRF, 24 to 36 hours after establishing a culture from cryopreserved cells. For subsequent subcultures, change the medium 48 hours after establishing the subculture.
2. Change the medium every other day thereafter, until the culture is approximately 80% confluent.
3. Once the culture reaches 80% confluence, change the medium every day.

Notes: *To achieve the highest cell densities, the culture medium should be changed every day as the cultures approach confluence. For rapidly proliferating subcultures, Corning® HUVEC-2 cells should be sub-cultured before the culture becomes confluent. The number of subcultures (passages) that can be achieved will vary with the starting cell density and the methods employed by individual investigators.*

HUVEC-2 cells seeded at 2.5 x 10^3 cells/cm^2 from cryopreserved cells should reach 80% confluence in 6-7 days.

Subculture of HUVEC-2 Cells

View the culture under a microscope to ascertain the condition of the culture (i.e., confluence, mitotic activity). This protocol is designed for the subculture of one 25 cm^2 culture flask. If different sized culture vessels are to be used, reagent volumes should be adjusted accordingly.

1. Assemble subculture reagents and materials:
    - Medium 200 or Medium 200PRF supplemented with LSGS or LSGS Kit
    - Trypsin/EDTA solution (Cascade Biologics' Cat. No. R-001-100)
    - Trypsin Neutralizer solution (Cascade Biologics' Cat. No. R-002-100)
    - Culture vessels (not provided)
    - Sterile pipettes (not provided)
    - Sterile 15 ml conical tubes (not provided)

Note: *We do NOT recommend warming the reagents prior to use.*

2. Remove all of the culture medium from the flask.
3. Add 4 ml of Trypsin/EDTA solution to the flask. Rock the flask to ensure that the entire surface is covered.
4. Immediately remove 3 ml of Trypsin/EDTA solution from the flask.
5. Incubate the flask at room temperature for 1-3 minutes.
6. View the culture under a microscope.
7. When the cells have become completely round, rap the flask gently to dislodge the cells from the surface of the flask.
8. Add 3 ml of Trypsin Neutralizer solution to the flask and transfer the detached cells to a sterile 15 ml conical tube.
9. Add 3 ml additional Trypsin Neutralizer solution to the flask and pipette the solution over the flask surface several times to remove any remaining cells. Add this solution to the 15 ml conical tube.
10. Centrifuge the cells at 180 x g for 7 minutes. Observe the cell pellet.
11. Remove the supernatant from the tube, being careful not to dislodge the cell pellet.
12. Resuspend the cell pellet in 4 ml supplemented Medium 200 or Medium 200PRF. Pipette the cells up and down with a 10 ml pipette to ensure a homogeneous cell suspension.
13. Determine the concentration of cells in the suspension.
14. Dilute the cells in supplemented Medium 200 or Medium 200PRF and seed new culture vessels with 2.5 x 10^3 cells/cm^2.
15. Incubate the cultures in a 37°C, 5% CO_2/95% air, humidified cell culture incubator.

Notes: *Damage to cultured HUVEC-2 cells can occur during trypsinization. This damage may result from exposure of the cells to the Trypsin/EDTA solution for excessive lengths of time, trypsinization at temperatures higher than 20°C and/or excessive mechanical agitation. Check to make sure that the temperature of trypsinization is appropriate and, if necessary, alter the incubation time of the procedure. Another common source of damage is centrifugation at excessive g forces. Check to make sure that the speed of the centrifuge is appropriate. One manifestation of cellular damage that may be evident after centrifugation is strings of cells (and debris) that do not pellet in the bottom of the tube. This is due to the presence of DNA from lysed cells in the solution. If this condition exists, the cell pellet may be lost upon aspiration of the supernatant containing the DNA strings. In many cases, viable cells can be rescued by pipeting the cells (and DNA) up and down in a 10 ml pipette to shear the DNA, and centrifuging the suspension again to recover the cells.*

Note: We do NOT recommend refreezing the HUVEC-2 cells after expansion as the cryopreservation process may result in altered growth performance of the cells.

Quality Control

Cells from which this lot was derived have been tested using immunohistochemical methods for the presence of von Willebrand factor (vWf) and CD31 antigen and for the absence of α-actin. The uptake of DiI-Ac-LDL was also confirmed. In addition, an independent laboratory tested cells from the same donor for the presence of Hepatitis B, Hepatitis C, and HIV-1 viruses. These agents were not detected. Each lot of HUVEC-2 cells has been tested by an independent laboratory for the presence of mycoplasma by indirect staining and direct agar methods. Mycoplasma was not detected. Upon thawing, the cells should be ≥70% viable (by trypan blue exclusion).

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