Human Microvascular Endothelial Cells, neonatal dermis (HMVECnd)

Catalog Number C-010-5C

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Product description

HMVECnd are human microvascular endothelial cells isolated from neonatal dermis. Each vial of this product contains ≥5 × 10^5 viable cells that are cryopreserved at the end of the tertiary culture stage in a medium containing 10% DMSO. Each lot of cells is tested using immunohistochemical methods for the presence of von Willebrand factor (vWf), CD31 antigen, and CD36 antigen and for the absence of α-actin. The uptake of DiI-Ac-LDL is also confirmed. An independent laboratory tests the cells for the presence of Hepatitis B, Hepatitis C, and HIV-1 viruses and mycoplasma. These agents were not detected. In our laboratory, each lot of cells is performance tested by culturing the cells through multiple passages in Medium 131 supplemented with Microvascular Growth Supplement (MVGS) in the absence of antibiotics and antimycotics. During this culture period, no contamination by bacteria, yeast, or fungi was detected. Upon thawing, the cells are guaranteed to be ≥70% viable and to have a potential of ≥16 population doublings when handled according to the directions provided in this document. For recommended precautions for handling human cells, read the Caution statement.

Product	Catalog No.	Amount	Shipping	Storage
Human Microvascular Endothelial Cells, neonatal dermis (HMVECnd)	C-010-5C	1 vial (≥5 × 10^5 viable cells/vial)	Frozen on dry ice	Liquid nitrogen vapor phase

Intended Use

Cryopreserved HMVECnd are intended for use by researchers investigating the molecular and biochemical basis of various normal and disease processes. **This product is for research use only. Not for use in animals, humans, or diagnostic procedures.**

Storage and stability

Cryopreserved HMVECnd should arrive frozen on dry ice.If the cells are not to be used immediately, 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 2 years of storage at liquid nitrogen temperatures.

Caution

Although cryopreserved cells are tested for the presence of various hazardous agents, diagnostic tests are not necessarily 100% accurate. Human cells may harbor other known or unknown agents, or organisms which could be harmful to your health or cause fatal illness. Treat all human cells as potential pathogens. Wear protective clothing and eyewear. Practice appropriate disposal techniques for potentially pathogenic or biohazardous materials. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

Initiate cultures from cryopreserved cells

We recommend seeding cells recovered from cryopreservation at a density of 5.0×10^3 viable cells/cm^2. For example, four 25-cm^2 tissue culture flasks can usually be established from one vial containing $\geq 5 \times 10^5$ HMVECnd. The following procedure is a sample protocol for establishing cultures from the contents of one vial.

Note: We recommend using culture surfaces that have been coated with Attachment Factor (Cat. No. S-006-100) before use. Refer to the instructions provided with the Attachment Factor for coating culture surfaces.

1. Prepare a bottle of supplemented Medium 131 (Cat. No. M-131-500) according to the instructions supplied with that product.
2. Remove the vial of cells to be thawed from liquid nitrogen and rapidly thaw by placing at 37°C in a water bath with gentle agitation for 1–2 minutes (or once a sliver of ice is left in the tube). Complete thawing can be detrimental to the cell viability.
3. 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.
4. Open the vial and pipet the suspension up and down with a 1-mL pipette to disperse the cells.
5. Remove 20 µL from the vial and dilute the cell suspension in 20 µL of trypan blue solution (Cat. No. 15250-061).
6. Using a hemacytometer, determine the number of viable cells per mL.
7. Dilute the contents of the vial (1 mL) to a concentration of 2.5×10^4 viable cells/mL using the supplemented medium from step 1, above.
8. Add 5 mL of cell suspension to each Attachment Factor-coated 25-cm^2 culture flask.
9. Following inoculation, swirl the medium in the flasks to distribute the cells. HMVECnd attach to culture surfaces quickly, and if the medium is not distributed immediately following inoculation, the cells may grow in uneven patterns.
10. 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.

Maintaining Stock Cultures

1. Change the culture medium to freshly supplemented Medium 131, 24 to 36 hours after establishing a secondary 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. We recommend subculturing the cells once the culture reaches 80% confluence. However, if cell densities in excess of 80% are desired, change the medium every day once the cells exceed 80% confluence.

Notes

- To achieve the highest cell densities, change the culture medium every day as the cultures approach confluence. For rapidly proliferating subcultures, subculture HMVECnd before the culture becomes confluent. The number of subcultures (passages) that can be achieved varies with the starting cell density and the methods employed.
- HMVECnd cultures seeded at 5.0×10^3 cells/cm^2 from cryopreserved cells should reach 80% confluence in 5–6 days. In this culture, most of the cells should have an epithelioid morphology, and be associated with each other in colonies. Some irregularly sized and shaped cells may be observed.

Subculture HMVECnd

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 used, adjust the reagent volumes accordingly.

1. Assemble subculture reagents and materials:
    - Medium 131 supplemented with MVGS
    - Trypsin/EDTA solution (Cat. No. R-001-100)
    - Trypsin Neutralizer solution (Cat. No. R-002-100)
    - Culture vessels (not provided)
    - Sterile pipettes (not provided)
    - Sterile 15-mL conical tubes (not provided)

Note: Do **not** warm the reagents prior to use.

2. Prepare new culture vessels by coating with Attachment Factor (provided with Medium 131) per the instructions included with the product.
3. Remove all of the culture medium from the flask.
4. Add 3 mL of Trypsin/EDTA solution to the flask. Rock the flask to ensure that the entire surface is covered.
5. Immediately remove all 3 mL of the Trypsin/EDTA solution.
6. Add 1 mL of fresh Trypsin/EDTA solution to the flask.
7. View the culture under a microscope. Incubate the cells at room temperature until the cells have become completely round, approximately 4–6 minutes.
8. Rap the flask gently to dislodge the cells from the surface of the flask.
9. Add 3 mL of Trypsin Neutralizer solution to the flask and transfer the detached cells to a sterile 15-mL conical tube.
10. Add 3 mL additional Trypsin Neutralizer solution to the flask and pipet the solution over the flask surface several times to remove any remaining cells. Add this solution to the 15-mL conical tube.
11. Centrifuge the cells at 180 × *g* for 7 minutes. Observe the cell pellet.
12. Remove the supernatant from the tube, being careful not to dislodge the cell pellet.
13. Resuspend the cell pellet in 4 mL supplemented Medium 131. Pipet the cells up and down with a 10-mL pipette to ensure a homogeneous cell suspension.
14. Determine the concentration of cells in the suspension.
15. Dilute the cells in supplemented Medium 131 and seed new culture vessels with 5.0×10^3 cells/cm^2.
16. Incubate the cultures in a 37°C, 5% CO_2/95% air, humidified cell culture incubator.

Notes

- Damage to cultured HMVECnd can occur during trypsinization. This damage can result from exposure of the cells to the Trypsin/EDTA solution for excessive lengths of time, trypsinization at temperatures exceeding room temperature, 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 at 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 can be lost upon aspiration of the supernatant containing the DNA strings. In many cases, viable cells can be rescued by pipetting the cells (and DNA) up and down with a 10-mL pipette to shear the DNA, and centrifuging the suspension again.

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