Cascade Biologics™
invitrogen cell culture

GIBCO®
invitrogen cell culture

Human Microvascular Endothelial Cells, neonatal dermis (HMVECnd)

Cat. no. C-010-5C

Quantity: ≥500,000 viable cells/vial

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 to be taken when handling human cells, please read the caution statement.

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.

Initiating 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 ≥5×10^5 HMVECnd. The procedure given below 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. Please 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 a vial of HMVECnd from liquid nitrogen storage, taking care to protect hands and eyes.
3. Dip the lower half of the vial into a 37ºC water bath to thaw.
4. 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.
5. Open the vial and pipet the suspension up and down with a 1 ml pipette to disperse the cells.
6. Remove 20 µl from the vial and dilute the cell suspension in 20 µl of trypan blue solution (Cat. no. 15250-061).
7. Using a hemacytometer, determine the number of viable cells per ml.
8. 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.
9. Add 5 ml of cell suspension to each Attachment Factor-coated 25 cm^2 culture flask.
10. 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.
11. Incubate the cultures in a 37ºC, 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% confluency. However, if cell densities in excess of 80% are desired, change the medium every day once the cells exceed 80% confluency.

Notes

- To achieve the highest cell densities, change the culture medium every day as the cultures approach confluency. For rapidly proliferating subcultures, subculture HMVECnd 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.
- HMVECnd cultures seeded at 5.0×10^3 cells/cm^2 from cryopreserved cells should reach 80% confluency 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.

Subculturing of 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 37ºC, 5% CO_2/95% air, humidified cell culture incubator.

Notes

- Damage to cultured HMVECnd 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 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 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 pipetting the cells (and DNA) up and down in a 10 ml pipette to shear the DNA, and centrifuging the suspension again.

Limited Use Label License No. 5: Invitrogen Technology

The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes. The buyer may transfer information or materials made through the use of this product to a scientific collaborator, provided that such transfer is not for any Commercial Purpose, and that such collaborator agrees in writing (a) not to transfer such materials to any third party, and (b) to use such transferred materials and/or information solely for research and not for Commercial Purposes. Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research. For products that are subject to multiple limited use label licenses, the terms of the most restrictive limited use label license shall control. Life Technologies Corporation will not assert a claim against the buyer of infringement of patents owned or controlled by Life Technologies Corporation which cover this product based upon the manufacture, use or sale of a therapeutic, clinical diagnostic, vaccine or prophylactic product developed in research by the buyer in which this product or its components was employed, provided that neither this product nor any of its components was used in the manufacture of such product. If the purchaser is not willing to accept the limitations of this limited use statement, Life Technologies is willing to accept return of the product with a full refund. For information on purchasing a license to this product for purposes other than research, contact Licensing Department, Life Technologies Corporation, 5791 Van Allen Way, Carlsbad, California 92008. Phone (760) 603-7200. Fax (760) 602-6500. Email: outlicensing@invitrogen.com.

For research use only. Not intended for any animal or human therapeutic or diagnostic use.