Human Aortic Endothelial Cells (HAEC)

Catalog Numbers C0065C

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Product description

HAEC are human aortic endothelial cells. Each vial of this product contains ≥5 × 10^5 viable cells that have been 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) and CD31 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 mycoplasma, Hepatitis B, Hepatitis C, and HIV-1 viruses. These agents were not detected. In our laboratory, each lot of cells is performance tested by culturing the cells through multiple passages in Human Large Vessel Endothelial Cell Basal Medium supplemented with Large Vessel Endothelial Supplement (LVES) 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.

Contents and storage

Contents	Cat. No.	Amount	Storage
Human Aortic Endothelial Cells (HAEC)	C0065C	1 vial (≥5 × 10^5 viable cells/vial)	Liquid nitrogen vapor phase

Note: Cryopreserved HAEC 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 2 years of storage at liquid nitrogen temperatures.

Required materials not supplied

Item	Source
Human Large Vessel Endothelial Cell Basal Medium or Human Large Vessel Endothelial Cell Basal Medium, Phenol Red Free	M-200-500 M-200PRF-500
Large Vessel Endothelial Supplement (LVES)	A1460801
Trypsin/EDTA Solution (TE)	R001100
Trypsin Neutralizer Solution	R002100
Trypan Blue Solution, 0.4%	15250061

Intended use

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

CAUTION! Although cryopreserved cells 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. 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 2.5×10^3 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 $>5 \times 10^5$ HAEC. The procedure given below is a sample protocol for establishing cultures from the contents of one vial.

1. Prepare a bottle of supplemented Human Large Vessel Endothelial Cell Basal Medium (Cat. No. M-200-500) or Human Large Vessel Endothelial Cell Basal Medium, Phenol Red Free (Cat. No. M-200PRF-500) according to the instructions that accompany that product.

2. Remove the vial of cells to be thawed from liquid nitrogen, then 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).

   Note: 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 pipette 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 (for example, Sigma Cat. No. T8154).

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 1.25×10^4 viable cells/mL using the supplemented medium from step 1.

8. 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.

9. Following inoculation, swirl the medium in the flasks to distribute the cells.

   Note: HAEC 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.

Maintain stock cultures

1. Change the culture medium to freshly supplemented Human Large Vessel Endothelial Cell Basal Medium or Human Large Vessel Endothelial Cell Basal Medium, Phenol Red Free, 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% confluency, change the medium every day.

Note:

- To achieve the highest cell densities, the culture medium should be changed every day as the cultures approach confluence. For rapidly proliferating subcultures, HAEC should be subcultured before the culture becomes confluent. The number of subcultures (passages) that can be achieved varies with the starting cell density and the methods employed.
- HAEC cultures seeded at 2.5×10^3 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 HAEC

Prepare for subculture

1. Assemble subculture reagents and materials:
   - Human Large Vessel Endothelial Cell Basal Medium or Human Large Vessel Endothelial Cell Basal Medium, Phenol Red Free supplemented with LVES
   - 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: It is NOT recommended to warm the reagents prior to use.

2. Remove all of the culture medium from the flask.

Add and remove Trypsin/EDTA Solution, then incubate

1. Add 4 mL of Trypsin/EDTA Solution to the flask. Rock the flask to ensure that the entire surface is covered.

2. Immediately remove 3 mL of Trypsin/EDTA Solution from the flask.

3. Incubate the flask at room temperature for 1-3 minutes.

View under microscope, add neutralizer, then centrifuge

1. View the culture under a microscope.
2. When the cells have become completely round, rap the flask gently to dislodge the cells from the surface of the flask.
3. Add 3 mL of Trypsin Neutralizer Solution to the flask and transfer the detached cells to a sterile 15 mL conical tube.
4. 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.
5. Centrifuge the cells at 180 × *g* for 7 minutes. Observe the cell pellet.
6. Remove the supernatant from the tube, being careful not to dislodge the cell pellet.

Resuspend, dilute, then incubate

1. Resuspend the cell pellet in 4 mL supplemented Human Large Vessel Endothelial Cell Basal Medium or Human Large Vessel Endothelial Cell Basal Medium, Phenol Red Free. Pipette the cells up and down with a 10 mL pipette to ensure a homogeneous cell suspension.
2. Determine the concentration of cells in the suspension.
3. Dilute the cells in supplemented Human Large Vessel Endothelial Cell Basal Medium or Human Large Vessel Endothelial Cell Basal Medium, Phenol Red Free and seed new culture vessels with 2.5×10^3 cells/cm^2.
4. Incubate the cultures in a 37°C, 5% CO_2/95% air, humidified cell culture incubator.

Troubleshooting

Observation	Probable cause	Recommended solution
Damage to cultured HAEC	Damage 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.	Ensure the temperature of trypsinization is appropriate and, if needed, alter the incubation time of the procedure.
	Damage can occur during centrifugation at excessive *g* forces.	Ensure the speed of the centrifuge is appropriate.
	Damage may be evident after centrifugation from 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.	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 to recover the cells.

Limited product warranty

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

The information in this guide is subject to change without notice.

Revision history: Pub. No. MAN0001575

Revision	Date	Description
4.0	12 August 2021	• Low Serum Growth Supplement (LSGS) replaced with Large Vessel Endothelial Supplement (LVES). • Medium 200 and Medium 200PRF rebranded to Human Large Vessel Endothelial Cell Basal Medium and Human Large Vessel Endothelial Cell Basal Medium, Phenol Red Free.
3.0	4 November 2019	Updated branding and storage conditions.
2.0	19 June 2009	Added Gibco logo, Invitrogen LULL, and contact information.
1.0	14 March 2009	New document.