Human Mammary Epithelial Cells (HMEC)

Catalog Number A10565

Pub. No. MAN0001573

Rev. 3.0

Product description

HMEC are normal human mammary epithelial cells isolated from reduction mammoplasty tissue. Each vial of this product contains ≥5 × 10^5 viable cells are cryopreserved at the end of the 6' culture in a medium containing 10% DMSO. Approved lots of cells have tested positive by immunofluorescent methods for cytokeratins 5/6, 8, 18, and E-cadherin. An independent laboratory tests the cells for the presence of mycoplasma, Hepatitis B, Hepatitis C, and HIV-1 viruses. These agents were not detected. Each lot of cells is performance tested by culturing the cells through multiple passages in complete HuMEC medium in the absence of antibiotics and antimycotics. During this culture period, no contamination by bacteria, yeast, or other fungi was detected. Upon thawing, the cells are guaranteed to be ≥70% viable (by trypan blue staining) and 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 Mammary Epithelial Cells (HMEC)	A10565	1 vial (≥5 × 10^5 viable cells/vial)	Frozen on dry ice	Liquid nitrogen vapor phase

Intended use

Cryopreserved HMEC 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 intended for human or animal therapeutic or diagnostic use.**

Storage and stability

Cryopreserved HMEC 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 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.

Required materials not supplied

Product	Cat. No./(Part No.)	Size
HuMEC Ready Medium	12752-010	1 kit
Contains:		
HuMEC Basal Serum Free Medium	(12753-018)	1 × 500 mL
HuMEC Supplement	(12754-016)	1 × 5 mL
Bovine Pituitary Extract	(13028-014)	1 × 25 mg
Trypsin/EDTA Solution (TE)	R-001-100	100 mL
Trypsin Neutralizer Solution	R-002-100	100 mL
Trypan Blue Solution, 0.4%	15250-061	100 mL

Initiate cultures from cryopreserved cells

We recommend seeding HMEC 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 are usually established from one vial containing $\geq 5 \times 10^5$ HMEC. The following procedure is a sample protocol for establishing cultures from one vial.

1. Prepare a bottle of supplemented HuMEC medium 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 just 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 gently 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 (for example, Invitrogen™ Trypan Blue Solution, 0.4%, Cat. No. 15250-061).
6. Use a hemacytometer to determine the number of viable cells per mL.

   Note: Do not warm the reagents prior to use.
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, above.
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 evenly distribute the cells.
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 18 hours after the culture has been initiated.

Maintain stock cultures

1. Change the culture medium to freshly supplemented medium, 18 to 24 hours following plating of 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 50% confluent.
3. Once the culture reaches 50% confluence, change the medium every day until the culture is approximately 80–90% confluent.

Notes

- To achieve the highest cell densities, change the culture medium every day as the cultures approach confluence. To obtain rapidly proliferating subcultures, subculture HMEC before they become more than 90% confluent. The number of subcultures (passages) that can be achieved varies with the starting cell density and the methods employed.
- HMEC cultures seeded at 2.5×10^3 cells/cm^2 from cryopreserved cells should reach 80–90% confluence in 5–7 days. At this time, most of the cells should have epithelial or "cobblestone" morphology. Some irregularly sized and shaped cells may be observed.

Subculture HMEC

Observe the culture under the microscope to confirm that it is subconfluent (80–90%), and that there are mitotic cells present. 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 supplemented medium and subculture reagents as described on page 2.

   Note: Do not warm the reagents prior to use.
2. Assemble the appropriate culture vessels, sterile pipettes, and sterile 15-mL conical tubes.
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 Trypsin/EDTA solution from the flask.
6. Add 1 mL of fresh Trypsin/EDTA solution to the flask.
7. Incubate the flask at 37ºC for 4–6 minutes. Observe the culture under a microscope to confirm cells have become round.
8. Rap the flask very gently to dislodge 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 tube.
10. Add 3 mL of additional Trypsin Neutralizer solution to the flask and pipette the solution over the flask surfaces several times to remove any remaining cells. Add this suspension 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. Pipet the cells up and down with a 10-mL pipette to ensure a homogeneous cell suspension.
14. Use a hemacytometer to determine the number of viable cells per mL.
15. Dilute the cells in supplemented medium and seed new culture vessels at 2.5 × 10^3 viable cells/cm^2.
16. Incubate the cultures in a 37°C, 5% CO_2/95% air, humidified cell culture incubator.

Notes

- Damage to cultured HMEC can occur during trypsinization. This damage can result from exposure of the cells to the Trypsin/EDTA solution for excessive lengths of time and/or excessive mechanical agitation. HMEC cultures can alternatively be trypsinized at room temperature incubation for 10–12 minutes. Monitor cell detachment and otherwise follow "Subculture HMEC".
- Invitrogen™ TrypLE™ Express (Cat. No. 12604-013) can be substituted for Trypsin/EDTA without affecting HMEC performance.
- Invitrogen™ Penicillin/Streptomycin (Cat. No.15140-148), and Gentamicin/Amphotericin (Cat. No. R-015-10) reagents have been performance validated for routine use and subculture with HMEC.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.htmL**. If you have any questions, please contact Life Technologies at **thermofisher.com/support**.

Life Technologies Corporation | 3175 Staley Road | Grand Island, NY 14072

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.

DISCLAIMER: TO THE EXTENT ALLOWED BY LAW, THERMO FISHER SCIENTIFIC INC. AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Important Licensing Information: This product may be covered by one or more Limited Use Label Licenses. By use of this product, you accept the terms and conditions of all applicable Limited Use Label Licenses.

thermofisher.com/support | thermofisher.com/askaquestion

thermofisher.com

23 October 2019