Rev. 0

Human Gastric Microvascular Endothelial Cell (HGMEC)

Catalog #2800

Cell Specification

Human Gastric Microvascular Endothelial Cells (HGMEC) form the innermost lining of small blood vessels within the gastric mucosa, serving as a vital barrier between the bloodstream and the tissue microenvironment while actively regulating blood flow, oxygen transport, and the synthesis of active biological factors [1,2]. Their research importance in gastric cancer is profound, as they drive tumor angiogenesis, growth, and metastasis through complex bidirectional crosstalk with gastric carcinoma cells, where vascular endothelial growth factor receptor plays a central regulatory role [1,3,4]. Investigating these interactions is critical for understanding how tumors build their vascular supply and for developing targeted anti-angiogenic therapies [3-5]. For research use, primary HGMECs provide stable *in vitro* models for investigating tumor-endothelial interactions, microvessel stabilization, and the mechanisms underlying gastric cancer angiogenesis and mucosal injury [4,5].

HGMEC from ScienCell Research Laboratories are isolated from human gastric tissue. HGMEC are cryopreserved at passage one and delivered frozen. Each vial contains >5 x 10^5 cells in 1 ml volume. HGMEC are characterized by immunofluorescence with antibodies specific to vWF/Factor VIII and/or CD31 (PECAM1). HGMEC are negative for HIV-1, HBV, HCV, mycoplasma, bacteria, yeast, and fungi. HGMEC are guaranteed to further culture under the conditions provided by ScienCell Research Laboratories; however, *HGMECs are not recommended for expanding or long-term cultures due to limited expansion capacity*.

Recommended Medium

It is recommended to use Endothelial Cell Medium (ECM, Cat. #1001) for culturing HGMEC *in vitro*.

Product Use

HGMEC are for research use only. They are not approved for human or animal use, or for application in *in vitro* diagnostic procedures.

Storage

Upon receiving, directly and immediately transfer the cells from dry ice to liquid nitrogen and keep the cells in liquid nitrogen until they are needed for experiments.

Shipping

Dry ice.

References

[1] Park, J. S., & Cresci, G. A. (2024). Dysfunctional intestinal microvascular endothelial cells: Insights and therapeutic implications in gastrointestinal inflammation. *Immunometabolism (Cobham, Surrey)*, *6*(2), e00043.

[2] Hull, M. A., Hewett, P. W., Brough, J. L., & Hawkey, C. J. (1996). Isolation and culture of human gastric endothelial cells. *Gastroenterology*, *111*(5), 1230-1240.

[3] Ren, J., Dong, L., Xu, C. B., & Pan, B. R. (2002). The role of KDR in the interactions between human gastric carcinoma cell and vascular endothelial cell. *World Journal of Gastroenterology*, *8*(4), 596.

[4] Caruso, R. A., Fedele, F., Finocchiaro, G., Pizzi, G., Nunnari, M., Gitto, G., ... & Venuti, A. (2009). Ultrastructural descriptions of pericyte/endothelium peg-socket interdigitations in the microvasculature of human gastric carcinomas. *Anticancer research*, *29*(1), 449-453.

[5] S Tarnawski, A., Ahluwalia, A., & K Jones, M. (2012). The mechanisms of gastric mucosal injury: focus on microvascular endothelium as a key target. *Current medicinal chemistry*, *19*(1), 4-15.

Instructions for culturing primary cells

Caution: Cryopreserved primary cells are very delicate. Thaw the vial in a 37ºC water bath and return the cells to culture as quickly as possible with minimal handling! Do not centrifuge the cells after thawing as this can damage the cells.

Note: HGMEC are very sensitive cells and they are not expected to proliferate many times in culture. Experiments should be well organized before thawing the cells. It is recommended that HGMEC are used as early as possible for experiments after initial plating with minimal expansion. We do not recommend subculturing the cells, however, if subculture is inevitable, then follow the instructions below with special care and only subculture the cells once.

Initiating the culture:

Note: ScienCell primary cells must be cultured in a 37ºC, 5% CO_2 incubator. Cells are only warranted if ScienCell media and reagents are used and the recommended protocols are followed.

1. Prepare a fibronectin-coated culture vessel (2 µg/cm^2, T-75 flask is recommended). To obtain a 2 µg/cm^2 fibronectin-coated culture vessel, add 10 ml of sterile Dulbecco's phosphate buffered saline, Ca^{++}- and Mg^{++}-free (Cat. #0303) to a T-75 flask and then add 150 µl of fibronectin stock solution (Cat. #8248). Leave vessel in a 37ºC incubator overnight (or for at least 2 hours).
2. Prepare complete medium. Decontaminate the external surfaces of medium bottle and medium supplement tubes with 70% ethanol and transfer them to a sterile field. Aseptically transfer supplement to the basal medium with a pipette. Rinse the supplement tube with medium to recover the entire volume.
3. Aspirate the fibronectin solution and add 20 ml of complete medium to the culture vessel. The fibronectin solution can be reused twice. Leave the vessel in the sterile field and proceed to thaw the cryopreserved cells.
4. Place the frozen vial in a 37ºC water bath. Hold and rotate the vial gently until the contents completely thaw. Promptly remove the vial from the water bath, wipe it down with 70% ethanol, and transfer it to the sterile field.
5. Carefully remove the cap without touching the interior threads. Gently resuspend and dispense the contents of the vial into the equilibrated, fibronectin-coated culture vessel.

   Note: Dilution and centrifugation of cells after thawing are not recommended as these actions are more harmful to the cells than the effect of residual DMSO in the culture. It is also important that cells are plated in fibronectin-coated culture vessels to promote cell attachment.
6. Replace the cap or lid of the culture vessel and gently rock the vessel to distribute the cells evenly. Loosen cap, if necessary, to allow gas exchange.
7. Return the culture vessel to the incubator.
8. Do not disturb the culture for at least 16 hours after the culture has been initiated. Refresh culture medium the next day to remove residual DMSO and unattached cells.

Maintaining the culture:

1. Refresh supplemented culture medium the next morning after establishing a culture from cryopreserved cells.
2. Change the medium every three days thereafter, until the culture is approximately 70% confluent.
3. Once the culture reaches 70% confluency, change medium every other day until the culture is approximately 90% confluent.

Subculturing:

1. Subculture when the culture reaches 90% confluency.
2. Prepare fibronectin-coated culture vessels (2 μg/cm^2) one day before subculture.
3. Warm complete medium, trypsin/EDTA solution, 0.05% (T/E, Cat. #0183), T/E neutralization solution (TNS, Cat. #0113), and DPBS (Ca^{++}- and Mg^{++}-free, Cat. #0303) to **room temperature**. We do not recommend warming reagents and medium in a 37ºC water bath prior to use.
4. Rinse the cells with DPBS.
5. Add 8 ml DPBS and 2 ml 0.05% T/E solution (Cat. #0183) into flask (in the case of a T-75 flask). Gently rock the flask to ensure complete coverage of cells by T/E solution. Use a microscope to monitor the change in cell morphology.

   Note: We recommend using ScienCell's 0.05% T/E solution, which is optimized to minimize cell damage due to over trypsinization. If 0.25% T/E solution (Cat. #0103) is used, then 9.6 ml of DPBS and 0.4 ml of 0.25% T/E solution should be used.

   Caution: Do NOT use undiluted trypsin when subculturing primary cells.
6. During incubation, prepare a 50 ml conical centrifuge tube with 5 ml of fetal bovine serum (FBS, Cat. #0500).
7. Once the cells completely round up, transfer T/E solution from the flask to a 50 ml centrifuge tube (a small percent of cells may detach) and continue to incubate the flask at 37ºC for another minute (no solution in the flask at this time).
8. At the end of incubation, gently tap the side of the flask to dislodge cells from the surface. Check under a microscope to make sure that all cells detach.
9. Add 5 ml of TNS solution to the flask and transfer detached cells to the 50 ml centrifuge tube. Rinse the flask with another 5 ml of TNS to collect the residual cells.
10. Examine the flask under a microscope for a successful cell harvest by looking at the number of cells being left behind; there should be less than 5%.
11. Centrifuge the 50 ml centrifuge tube at 1000 rpm for 5 minutes. Gently resuspend cells in culture medium.
12. Count and plate cells in a new fibronectin-coated culture vessel with the recommended cell density. A seeding density of 8,000-10,000 cells/cm^2 is recommended.

    Note: We do not recommend cryopreservation of primary cells by the end user. Refreezing cells may damage them and affect cell performance. ScienCell does not guarantee primary cells cryopreserved by the end user.

Caution: Handling human derived products is potentially biohazardous. Although each cell strain tests negative for HIV, HBV and HCV DNA, diagnostic tests are not necessarily 100% accurate, therefore, proper precautions must be taken to avoid inadvertent exposure. Always wear gloves and safety glasses when working with these materials. Never mouth

pipette. We recommend following the universal procedures for handling products of human origin as the minimum precaution against contamination [1].

[1] Grizzle WE, Polt S. (1988) “Guidelines to avoid personal contamination by infective agents in research laboratories that use human tissues.” *J Tissue Cult Methods*. 11: 191-9.