ScienCell™
Research Laboratories

Human Dermal Lymphatic Endothelial Cells (HDLEC)

Catalog Number: 2010

Cell Specification

The lymphatic system is an essential part of the immune system. It serves distinct yet complementary function with the blood vascular system in maintaining tissue homeostasis. The lymphatic system returns fluid and macromolecules from the tissues back into blood circulation and, thus, plays a vital role in the regulation of fluid, protein, and pressure equilibrium in tissues [1]. Although lymphatic capillary endothelial cells have many properties in common with the endothelium of blood vessels, they also have distinct structural characteristics reflecting their specific functions. Lymphatic capillaries lack mural cells and are characterized by an incomplete or absent basement membrane [2]. Lymphatic endothelium typically contains numerous invaginations and cytoplasmic vesicles as well as characteristic overlapping intercellular junctions [3]. One of the most striking characteristics of the lymphatic capillary is its integration within the interstitium which is connected to the extracellular matrix by fine strands of elastic fibers.

HDLEC from ScienCell Research Laboratories are isolated from human dermal tissue. HDLEC are cryopreserved on passage two (immediately after purification) and delivered frozen. Each vial contains >5 x 10^5 cells in 1 ml volume. HDLEC are characterized by immunofluorescent method with antibodies to CD31, podoplanin and Lyve1. HDLEC are negative for HIV-1, HBV, HCV, mycoplasma, bacteria, yeast and fungi. HDLEC are guaranteed to further expand for 15 population doublings at the conditions provided by ScienCell Research Laboratories.

Recommended Medium

It is recommended to use Endothelial Cell Medium (ECM, Cat. No. 1001) for the culturing of HDLEC *in vitro*.

Product Use

HDLEC are for research use only. It is not approved for human or animal use, or for application in *in vitro* diagnostic procedures.

Storage

Directly and immediately transfer cells from dry ice to liquid nitrogen upon receiving and keep the cells in liquid nitrogen until cell culture needed for experiments.

Shipping

Dry ice.

Reference

[1] Swartz, A. and Skobe, M. (2001) Lymphatic function, lymphangiogenesis, and cancer metastasis. Microsc. *Res. Tech.* 55:92-99.

[2] Leak, L. V. (1970) Electron microscopic observations on lymphatic capillaries and the structural components of the connective tissue-lymph interface. *Microvasc. Res.* 2, 361-391

[3] Podgrabinska, S., Braun, P., Velasco, P., Kloos, B., Pepper, M., Jackson, D. and Skobe, M. (2002) Molecular characterization of lymphatic endothelial cells. *PNAS* 99:16069-16074.

Instruction for culturing cells

Caution: Cryopreserved cells are very delicate. Thaw the vial in a 37°C waterbath and return them to culture as quickly as possible with minimal handling!

Set up culture after receiving the order:

1. Prepare a fibronectin coated flask (2 μg/cm^2, T-75 flask is recommended). Add 10 ml of sterile Dulbecco's phosphate buffered saline (DPBS) to a T-75 flask and then add 150 μl of fibronectin stock solution (1 mg/ml, Sigma cat. no. F1141). Leave the flask in incubator overnight.
2. Prepare complete medium: decontaminate the external surfaces of medium and medium supplements with 70% ethanol and transfer them to sterile field. Aseptically open each supplement tube and add them to the basal medium with a pipette. Rinse each tube with medium to recover the entire volume.
3. Aspirate fibronectin solution and add 20 ml of complete medium to the flask. Leave the flask in the hood and go to thaw the cells. The fibronectin solution can be used twice.
4. Place the vial in a 37°C waterbath, hold and rotate the vial gently until the contents are completely thawed. Remove the vial from the waterbath immediately, wipe it dry, rinse the vial with 70% ethanol and transfer it to a sterile field. Remove the cap, being careful not to touch the interior threads with fingers. Using a 1 ml eppendorf pipette gently resuspend the contents of the vial.
5. Dispense the contents of the vial into the equilibrated, fibronectin-coated flask. A seeding density of 5,000 cells/cm^2 is recommended.
   Note: Dilution and centrifugation of cells after thawing are not recommended since these actions are more harmful to the cells than the effect of DMSO residue in the culture. It is also important that endothelial cells are plated in fibronectin coated flask that promotes cell attachment.
6. Replace the cap or cover of flask, and gently rock the flask to distribute the cells evenly. Loosen cap if necessary to permit gas exchange.
7. Return the culture vessels to the incubator.
8. For best results, do not disturb the culture for at least 16 hours after the culture has been initiated. Change the growth medium the next day to remove the residual DMSO and unattached cells, then every other day thereafter. A healthy culture will display cobblestone or spindle shaped morphology, nongranular cytoplasm and the cell number will be double after two to three days in culture.

Maintenance of Culture:

1. Change the medium to fresh supplemented medium the next morning after establishing a culture from cryopreserved cells. For subsequent subcultures, change 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 medium every day until the culture is approximately 90% confluent.

Subculture:

1. Subculture the cells when they are over 90% confluent.
2. Prepare fibronectin coated flasks (2 $\mu g/cm^2$) one day before subculture.
3. Warm medium, trypsin/EDTA solution, trypsin neutralization solution, and DPBS to **room temperature**. We do not recommend warming the reagents and medium at 37^oC waterbath prior to use.
4. Rinse the cells with DPBS.
5. Incubate cells with 10 ml of trypsin/EDTA solution (in the case of T-75 flask) until 80% of cells are rounded up (monitored with microscope). Add 10 ml of trypsin neutralization solution to the digestion immediately and gently rock the culture vessel.
   Note: Use ScienCell Research Laboratories' trypsin/EDTA solution that is optimized to minimize the killing of the cells by over trypsinization.
6. Harvest and transfer released cells into a 50 ml centrifuge tube. Rinse the flask with another 10 ml of growth medium to collect the residue cells. Examine the flask under microscope to make sure the harvesting is successful by looking at the number of cells left behind. There should be less than 5%.
7. Centrifuge the harvested cell suspension at 1000 rpm for 5 min and resuspend cells in growth medium.
8. Count cells and plate them in a new, fibronectin-coated flask with cell density as recommended.

Caution: Handling human derived products is potentially bioharzadous. Although each cell strain testes negative for HIV, HBV and HCV DNA, diagnostic tests are not necessarily 100% accurate, therefore, proper precautions mush be taken to avoid inadvertent exposure. Always wear gloves and safety glasses when working 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, W. E., and Polt, S. S. (1988) Guidelines to avoid personal contamination by infective agents in research laboratories that use human tissues. *J Tissue Culture Methods*. 11(4).