# Human Lymphatic Endothelial Cell (neonatal dermis)

**Catalog Number: 14-003**
**Size: 0.5 million cells/vial**

**Description:** Human lymphatic endothelial cells were isolated from human neonatal dermis. The cells were cryopreserved in a medium containing 10% DMSO and 90% FBS. Upon thawing, the cells can be expanded for at least 5 passages or 12 population doublings if using recommended media and following directions for handling. The cells are showed to be positive for CD31, podoplanin and another lymphatic marker Prox-1 by immunostaining with corresponding antibodies.

**Storage and Stability:** The cryopreserved cells will be shipped on dry ice. Upon arrival, if the cells are not to be used immediately, the vial should be stored in the vapor phase of a liquid nitrogen freezer. The cells are normally stable for years in liquid nitrogen.

**Recommended Medium System:** The cells grow optimally in medium EGM2-MV (from Cambrex, formerly Clonetics) supplemented with 20% off-clot human serum instead of FBS. It will grow in other media for microvascular endothelial cells; however, the lymphatic endothelial markers may be lost prematurely.

**Other Reagents Needed:** The following reagents are needed in addition to culture media for growing lymphatic endothelial cells:

1. 0.1% Gelatin in PBS to coat culture dish or other surface overnight;
2. 0.025% Trypsin and 0.01% EDTA in PBS pH7.4
3. PBS or serum free media for washing cells before adding Trypsin/EDTA

**Special Notes for Culture:**
1. Initiation of Culture: Thaw the vial in a 37$^{o}$C.water bath and transfer the cells to a centrifuge tube with 10 ml culture medium. Gently spin down the cells at 200g for 5 min to remove DMSO. Resuspend cells in fresh medium and plate in a 100 mm dish or T75 flask precoated with Gelatin. Change medium next day and then every 2 days. Split cells when they are about 80-90% confluence. Avoid letting cells reach complete confluence.

2. When splitting cells, wash cells first with PBS before adding the special Typsin/EDTA (reduced concentration) and incubate at room temperature (NOT 37$^{o}$C) for a minimal time (usually 3 min is enough) to lift all cells. Neutralize the trypsin by adding serum containing medium and centrifuge once. Cells are then resuspended and diluted 1:4 or 1:5 to new precoated culture dishes/flasks. Change medium next day and then every 2 days.

**Usage:** This product is for research use only. **Not for human use!**
**Caution:** Although these cells have been tested negative for common pathogens such as HIV-1, Hepatitis B and C. Diagnostic tests are not necessarily 100% accurate. Users should wear protective clothing and eyewear as they normally do when handling human cells. In no events should AngioBio be held liable to any consequence caused by any party misusing its products.

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