

User's Manual and Instructions

Human Endothelial Progenitor Outgrowth Cells

Catalog Number: Z7030001

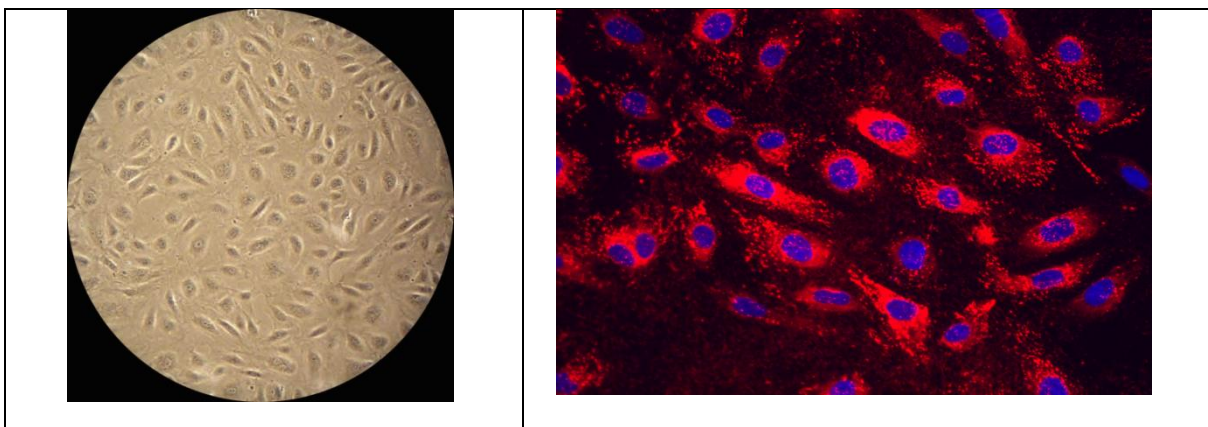
Introduction

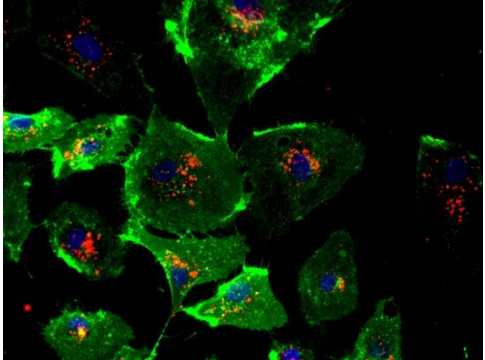
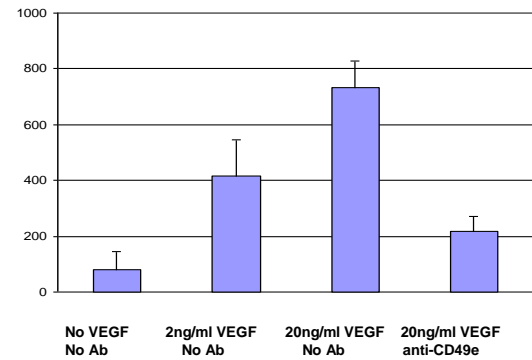
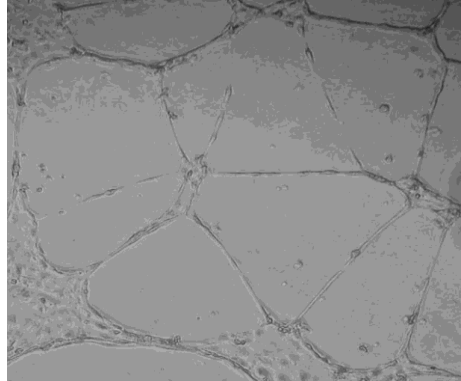
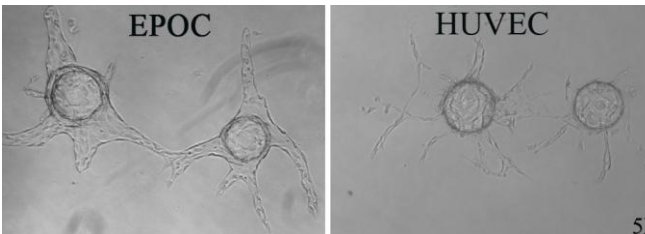
The endothelial progenitor cell (EPC) is a primitive cell type in the endothelial lineage. They are bone marrow originated cells with properties similar to those of embryonic angioblasts. These progenitor cells migrate into the blood stream and are capable of differentiating into a variety of mature vascular endothelial cell types.

EPC play an important role in both angiogenesis and vasculogenesis. Recent evidence suggests the involvement of EPC in tumor growth and metastasis. Change in EPC number has been associated with lymphoma, multiple myeloma, Lewis lung tumor, and hepatocellular carcinoma (HCC). Alteration in EPC number and function has also been observed in pathogenesis of a variety of diseases including coronary artery disease (CAD), ischemia, pulmonary hypertension, cerebral vascular disease, acute myocardial infarction, diabetes mellitus, arthritis, and wound healing. In addition, EPC have impact on aging and smoking-related diseases, suggesting potential uses of EPC in these areas.

Specification and Characterization of EPOC

BioChain's human endothelial progenitor outgrowth cells (hEPOC) are isolated from cord blood and further cultured in vitro. Our hEPOC product is delivered at the 4th passage, either as cryopreserved or proliferating cells. This cell is considered as late stage EPC because it is CD 133-. Each cryovial contains $>5 \times 10^5$ cells in 1 ml freezing medium. Our hEPOC have the cobblestone morphology common to endothelial cells. They are characterized by antigen expression (CD146, KDR, CD34), von Willebrand Factor immunostaining, LDL uptake and lectin binding, tube formation and migration assays. Human donors are negative for HIV-1, HBV or HCV. Donor information is available upon request.



<p>Figure 1. Morphology of cultured hEPOC. Passage 4, 100x.</p>	<p>Figure 2. Immunostaining of hEPOC with anti-von Willebrand factor polyclonal antibody (red). Cell nuclei were stained blue (DAPI). Passage 4, 400x</p>										
	 <table border="1"> <caption>Data for Figure 4: hEPOC migration in response to VEGF</caption> <thead> <tr> <th>Condition</th> <th>Approximate Cell Count</th> </tr> </thead> <tbody> <tr> <td>No VEGF No Ab</td> <td>100</td> </tr> <tr> <td>2ng/ml VEGF No Ab</td> <td>400</td> </tr> <tr> <td>20ng/ml VEGF No Ab</td> <td>750</td> </tr> <tr> <td>20ng/ml VEGF anti-CD49e</td> <td>200</td> </tr> </tbody> </table>	Condition	Approximate Cell Count	No VEGF No Ab	100	2ng/ml VEGF No Ab	400	20ng/ml VEGF No Ab	750	20ng/ml VEGF anti-CD49e	200
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<p>Figure 3: hEPOC are positive for both Dil-Ac-LDL uptake (red) and FITC-Ulex-Lectin binding (green). Nuclei are stained blue (DAPI). Passage 4, 400x</p>	<p>Figure 4: hEPOC migrate in response to VEGF. EPC were starved and migrated for 6 hours through 8 μm pores in a 24 well insert system. An antibody against α5 integrin was used as a control blocking antibody.</p>										
											
<p>Figure 5: hEPOC tube formation. hEPOC were seeded on a layer of Matrigel and allowed to form tube-like structures for 1 day. 40x</p>	<p>Figure 6: hEPOC in sprouting assay in the presence of fibroblast-conditioned media. Comparing with results from HUVEC at identical conditions, tube-like structures from EPC are much more robust and stable. 5X</p>										

Storage

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

Shipping

Cryopreserved cells are shipped on dry ice; proliferating cells are shipped at room temperature.

Instructions for culturing hEPOC

I. Preparation of EOPC Growth Medium

We recommend the use of BioChain's hEPOC growth medium (Cat# Z7030003) for culturing our hEPOC.

1. Thaw EPOC growth medium supplement (Cat# Z7030005) at room temperature.
2. Prepare hEPOC growth medium by adding the entire volume (25 ml) of hEPOC growth medium supplement to the bottle (500 ml) of hEPOC basal medium (Cat# Z7030004). BioChain's hEPOC growth medium does not contain antibiotics, but antibiotics may be added to the medium if contamination is a concern.
3. Prior to use, warm up a portion of the hEPOC growth medium in a 37°C water bath.

II. Thawing frozen cells

1. Warm hEPOC growth medium in a 37°C water bath.
2. Wipe the outside of the frozen vial with 70% ethanol. Quickly thaw the frozen cells in the water bath.
3. Add hEPOC growth medium to a culture vessel. Aseptically transfer the cell suspension to the culture vessel. Rinse the vial with 1 ml of growth medium; and combine the rinse with the cells. A seeding density of 10,000 to 12,500 cells/cm² is recommended. Centrifugation is more damaging to the cells than the effects of residual DMSO in the culture, and should NOT be performed to remove the cryoprotectant cocktail.
4. Incubate the cells at 37°C with 5% CO₂ and 95% air in a humidified incubator. Change medium the next day, then every other day thereafter. A healthy culture displays the cobblestone morphology and cell number should double after two to three days in culture.

III. Sub-culturing cells

1. Subculture the cells when they reach approx. 90% confluence.
2. Warm Dulbecco's PBS, 0.05% trypsin/EDTA and EPC growth medium in a 37°C water bath.
3. Rinse the cells with Dulbecco's PBS.
4. Incubate the cells with trypsin/EDTA solution until approx. 90% of the cells begin to detach. Monitor the cells with a microscope and avoid over-trypsinization.
5. Add fetal bovine serum equal to 1/10th volume of the trypsin/EDTA to neutralize trypsin, gently shake the culture vessel to mix.
6. Gently re-suspend the cells and transfer the cells into a 15ml conical tube.
7. Centrifuge the cell suspension at 150 x g for 5 minutes at room temperature.
8. Carefully remove the supernatant without disturbing the cell pellet. Re-suspend the cells in 2 ml growth medium.
9. Count cells and plate them in a new culture vessel at the density of approx. 10,000 to 12,500/cm².
10. Incubate the cells at 37°C with 5% CO₂ and 95% air in a humidified incubator. Change medium every other day.

Related Products

hEPOC Growth Medium (Cat# Z7030003).
hEPOC Basal Medium (Cat# Z7030004)
hEPOC Growth Medium Supplement (Cat# Z7030005)
hEPOC Freezing Medium (Cat# Z7030006)

References

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