

## User's Manual

**Product: Human Endothelial Progenitor Outgrowth Cells**

**Catalog Number: Z7030001/Z7030002**

### 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 EPC

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 4<sup>th</sup> 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.

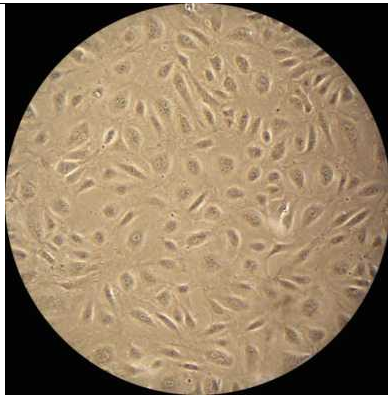


Figure 1. Morphology of cultured hEPOC. Passage 4, 100x.

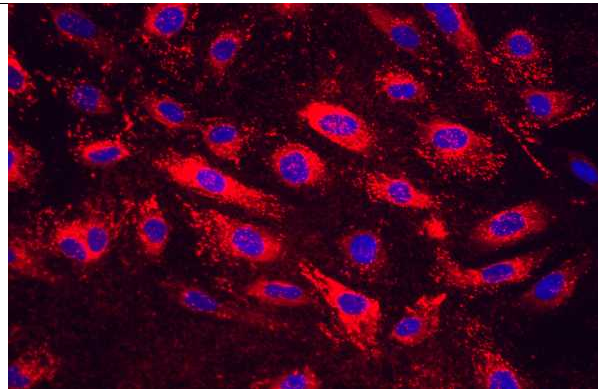


Figure 2. Immunostaining of hEPOC with anti-von Willebrand factor polyclonal antibody (red). Cell nuclei were stained blue (DAPI). Passage 4, 400x



#### UK & Rest of World

184 Milton Park, Abingdon  
OX14 4SE, Oxon, UK  
Tel: +44 (0) 1235 828 200  
Fax: +44 (0) 1235 820 482

#### Switzerland

Centro Nord-Sud 2E  
CH-6934 Bioggio-Lugano  
Tel: +41 (0) 91 604 55 22  
Fax: +41 (0) 91 605 17 85

#### Deutschland

Tel: +49 (0) 69 779099  
Fax: +49 (0) 69 13376880

**amsbio.com**

info@amsbio.com

AMS Biotechnology (Europe) Limited  
Registered in England & Wales No.2117791

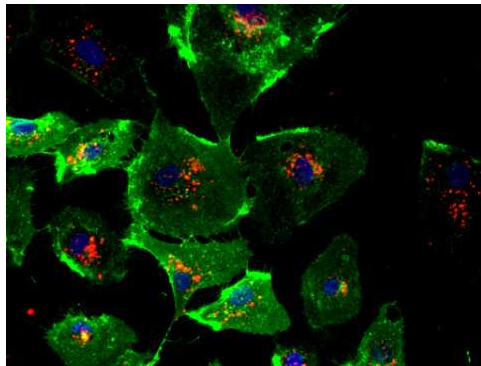


Figure 3: hEPOC are positive for both Dil-Ac-LDL uptake (red) and FITC-Ulex-Lectin binding (green). Nuclei are stained blue (DAPI). Passage 4, 400x

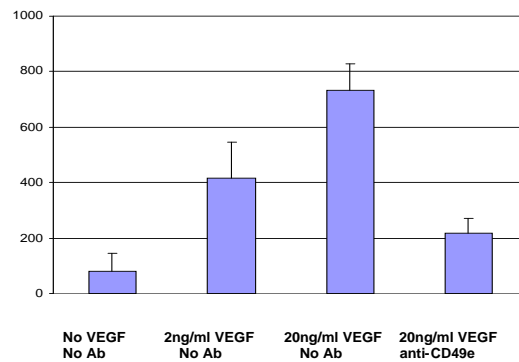


Figure 4: hEPOC migrate in response to VEGF. EPC were starved and migrated for 6 hours through 8  $\mu$ m pores in a 24 well insert system. An antibody against  $\alpha$ 5 integrin was used as a control blocking antibody.

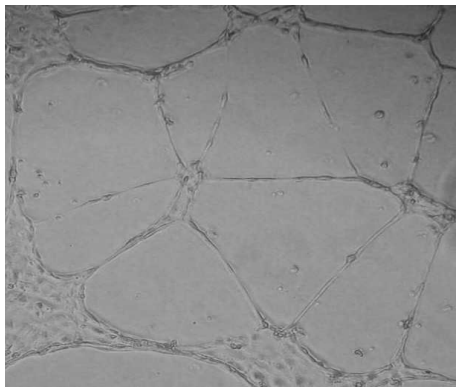


Figure 5: hEPOC tube formation. hEPOC were seeded on a layer of Matrigel and allowed to form tube-like structures for 1 day. 40x

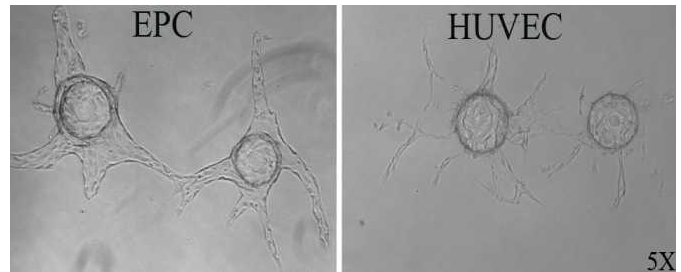


Figure 6: hEPOC in sprouting assay in the presence of fibroblast-conditioned media. Comparing with results from HUVEC at identical conditions, tube-like structures from EPC are much more robust and stable. 5X

### 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 EPC Growth Medium

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

1. Thaw EPC 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.



UK & Rest of World  
184 Milton Park, Abingdon  
OX14 4SE, Oxon, UK  
Tel: +44 (0) 1235 828 200  
Fax: +44 (0) 1235 820 482

Switzerland  
Centro Nord-Sud 2E  
CH-6934 Bioggio-Lugano  
Tel: +41 (0) 91 604 55 22  
Fax: +41 (0) 91 605 17 85

Deutschland  
Tel: +49 (0) 69 779099  
Fax: +49 (0) 69 13376880

amsbio.com  
info@amsbio.com

AMS Biotechnology (Europe) Limited  
Registered in England & Wales No.2117791

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/cm<sup>2</sup> 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% CO<sub>2</sub> 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 1/10<sup>th</sup> 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/cm<sup>2</sup>.
10. Incubate the cells at 37°C with 5% CO<sub>2</sub> and 95% air in a humidified incubator. Change medium every other day.

## IV. Proliferating hEPOC

Proliferating hEPOC are provided in T25, T75, T125, and T225 flask full of hEPOC Growth Medium. When the flask arrives:

1. Pour out most of the medium from the flask and leave appropriate amount in the flask.
2. Incubate the cells at 37°C with 5% CO<sub>2</sub> 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.

## Related Products

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

## References

1. Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, Witzenbichler B, Schatteman G and Isner JM (1997). Isolation of putative progenitor endothelial cells for angiogenesis. *Science*. 275:964-967.
2. Gupta M (2007). Circulating endothelial cells and circulating endothelial cell progenitors as surrogate markers for determining response to antiangiogenic agents. *Clin Colorectal Cancer* 6(5):337-338.



### UK & Rest of World

184 Milton Park, Abingdon  
 OX14 4SE, Oxon, UK  
 Tel: +44 (0) 1235 828 200  
 Fax: +44 (0) 1235 820 482

### Switzerland

Centro Nord-Sud 2E  
 CH-6934 Bioggio-Lugano  
 Tel: +41 (0) 91 604 55 22  
 Fax: +41 (0) 91 605 17 85

### Deutschland

Tel: +49 (0) 69 779099  
 Fax: +49 (0) 69 13376880

amsbio.com

info@amsbio.com

AMS Biotechnology (Europe) Limited  
 Registered in England & Wales No.2117791

3. Rafii S, Lyden D, Benezra R, Hattori K, and Heissig B (2002). Vascular and haematopoietic stem cells: novel targets for anti-angiogenesis therapy? *Nat Rev Cancer* 2:826-835.
4. Dzau JV, Gneccchi M, Pachori AS, Morello F, Melo LG (2005). Therapeutic potential of endothelial progenitor cells in cardiovascular diseases. *Hypertension* 46:7-18.
5. Grisar J, Aletaha D, Steiner CW, Kapral T, Steiner S, Seidinger D, Weigel G, Schwarzingler I, Wolozczuk W, Steiner G and Smolen JS (2005). Depletion of endothelial progenitor cells in the peripheral blood of patients with rheumatoid arthritis. *Circulation* 111:204-211.
6. Murasawa S and Asahara T (2005). Endothelial Progenitor Cells for Vasculogenesis. *Physiology* 20:36-42.
7. Suh W, Kim KL, Kim JM, et al., (2005). Transplantation of Endothelial Progenitor Cells Accelerates Dermal Wound Healing with Increased Recruitment of Monocytes/Macrophages and Neovascularization. *Stem Cells* 23:1571-1578.
8. Ho JWY, Pang RWC, Lau C, Sun CK, Yu WC, Fan ST, Poon RTP (2006). Significance of Circulating Endothelial Progenitor Cells in Hepatocellular Carcinoma *Hepatology* 44:836-843.

**UK & Rest of World**

184 Milton Park, Abingdon  
OX14 4SE, Oxon, UK  
Tel: +44 (0) 1235 828 200  
Fax: +44 (0) 1235 820 482

**Switzerland**

Centro Nord-Sud 2E  
CH-6934 Bioggio-Lugano  
Tel: +41 (0) 91 604 55 22  
Fax: +41 (0) 91 605 17 85

**Deutschland**

Tel: +49 (0) 69 779099  
Fax: +49 (0) 69 13376880

**amsbio.com****info@amsbio.com**

AMS Biotechnology (Europe) Limited  
Registered in England & Wales No.2117791