BioChain®
www.biochain.com
Tel: 1-888-762-2568 Fax: 1-510-783-5386 Email: info@biochain.com

User's Manual and Instructions

Mouse Endothelial Progenitor Outgrowth Cells

Catalog Number: Z7030031

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 plays 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.

Figure 1. Morphology of mEPOC in culture. Passage 9, 100x.

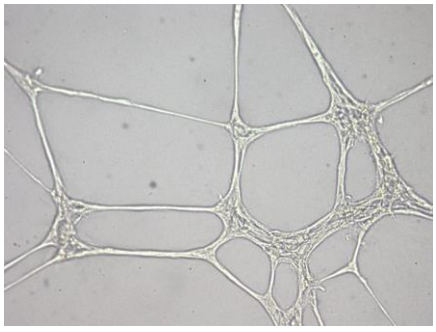

Figure 2. Mouse EPOC tube formation. Mouse EPCs were seeded on a layer of Matrigel and allowed to form tube-like structures for 18 hrs. 100x.

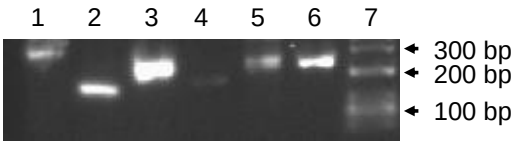

Figure 3. Mouse EPOCs express endothelial markers. Total RNA was extracted from mEPOCs with Dr. P Kit (Biochain). RT-PCR was conducted with Optimax first strand cDNA synthesis kit (Biochain). 1. mCD31 (300bp); 2. mCD105 (130 bp); 3. mGAPDH (200 bp);4. mVEGFR2 (160 bp); 5. mVEGFR1 (240 bp); 6. mNRP1 (240 bp); 7. 100 bp ladder (Biochain)

Specification and Characterization of EPOC

BioChain's mouse endothelial outgrowth cells (mEPOC) are isolated from bone marrow of 7-8 week-old C57/Bl6 mice and further cultured in vitro. Our mEPOC product is delivered at the 9-10th passage, either as cryopreserved or proliferating cells. The cell is considered as late stage EPC since it is CD 133-. Each cryovial contains >5 x 10^5 cells in 1 ml freezing medium. Our mEPOCs have the spindle morphology common to endothelial progenitor cells.

Tel: 1-888-762-2568 Fax: 1-510-783-5386 Email: info@biochain.com

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 EPOC

I. Preparation of mEPOC Growth Medium
We recommend the use of BioChain's mEPOC growth medium (Cat# Z7030033) for culturing our mEPC.
1. Thaw mEPOC growth medium supplement (Cat# Z7030034) in a water bath at room temperature.
2. Prepare mEPOC growth medium by adding the entire volume (25 ml) of EPOC growth medium supplement to the bottle (500 ml) of mEPOC basal medium (Cat# Z7030035). BioChain's mEPOC 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 mEPOC growth medium in a 37°C water bath.

II. Thawing frozen cells
1. Warm EPOC 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 at 37°C.
3. Aseptically transfer the cell suspension to a 15ml tube. Rinse the vial with 1 ml of growth medium; and combine the rinse with the cells in 15ml tube. Centrifuge at 170 g (or 1400 rpm) for 5 minutes to precipitate the cells. Remove the supernatant. Add 5 ml of fresh mouse EPC medium and put into a T25 flask.
4. Incubate the cells at 37°C with 5% CO2 and 95% air in a humidified incubator. Do NOT change the medium for the first 3 days. After the initial 3 day incubation period, change medium. Then change the medium every other day. A healthy culture displays a spindle morphology and cell number should double after two to three days in culture.

III. Sub-culturing cells
1. Subculture the cells when they are close to 100% confluence.
2. Change medium the day before passing the cells.
3. Warm Dulbecco's PBS, 0.05% trypspin/EDTA and mEPOC growth medium in a 37°C water bath.
4. Rinse the cells with Dulbecco's PBS.
5. Incubate the cells with trypsin/EDTA solution (1.5 ml/25 cm^2) 3-5 minutes until approx. 90% of the cells begin to detach. Pepitting and gently padding the vessel to detach the cells.
6. Add fetal bovine serum equal to 1/10th volume of the trypsin/EDTA to neutralize trypsin, gently shake the culture vessel to mix.
7. Gently re-suspend the cells and transfer the cells into a 15 ml conical tube.
8. Centrifuge the cell suspension at 170 x g for 5 minutes at room temperature.
9. Carefully remove the supernatant without disturbing the cell pellet. Re-suspend the cells in growth medium.
10. Plate them in new culture vessels in 1:5 ratio.
11. Incubate the cells at 37°C with 5% CO2 and 95% air in a humidified incubator. Change medium every other day.

IV. Proliferating EPOC
Proliferating EPOC are provided in T25, T75, T125, and T225 flask full of mEPOC 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% CO2 and 95% air in a humidified incubator. Change medium the next day, then every other day thereafter. A healthy culture displays the spindle morphology and cell number should double after two to three days in culture.

Tel: 1-888-762-2568 Fax: 1-510-783-5386 Email: info@biochain.com

Related Products
mEPOC Growth Medium (Cat# Z7030033)
mEPOC Basal Medium (Cat# Z7030035)
mEPOC Growth Medium Supplement (Cat# Z7030034)
mEPOC Freezing Medium (Cat# Z7030036)

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.
3. Gao D, Nolan DJ, Mellick AS, Bambino K, McDonnell K, Mittal V. Endothelial progenitor cells control the angiogenic switch in mouse lung metastasis. Science. 2008 Jan 11; 319(5860):195-8.