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User's Manual and Instructions

Human CD133+ Endothelial Progenitor Cells

Catalog Number: Z7030071

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.

Early stage EPCs are CD133 and CD34 double positive. They show significant differences from late stage EPCs and ECs in term of functions and cell surface marker expression. CD133+ EPCs are maintained in suspension culture and can differentiate into CD133 negative late stage endothelial progenitor cells, such as Biochain's endothelial progenitor outgrowth cells, and endothelial cells when cultured in endothelial culture medium (Gehling et al., 2000; Quirici et al., 2001).

EPCs play an important role in both angiogenesis and vasculogenesis. CD133 positive EPC had wide application for tissue regeneration and cell based therapies in human diseases. They have been used to treat ischemia, cardiovascular disease, diabetes and renal disease (Rafii & Lyden 2003; Kawamoto & Losordo 2008; Mizrak et al.2008).

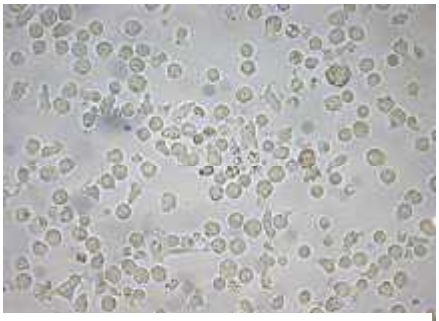

Figure 1. Morphology of CD133+ EPC in culture. Passage 7, 400x.

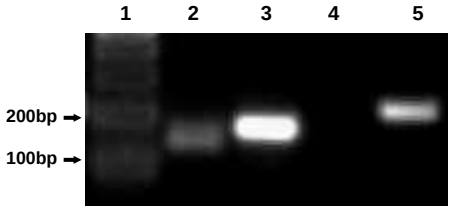

Figure 2. Cord Blood derived hEPCs are CD133 and CD34 double positive. Total RNA was extracted from hEPCs with Dr.P Kit (Biochain). RT-PCR was conducted with Optimax first strand cDNA synthesis kit (Biochain). 1. 100bp ladder (BioChain). 2. CD133. 3. CD34. 4. GAPDH (no RT) 5. GAPDH.

Recent evidence suggests the involvement of EPC in tumor growth and metastasis (Gao et al. 2008). 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, EPCs have impact on aging and smoking-related diseases, suggesting potential usage of EPC in these areas.

Specification and Characterization of EPC

BioChain's CD133+ EPC cells are isolated from human umbilical cord blood and further cultured in vitro. Our EPC product is delivered at the 3rd passage as cryopreserved cells. Each cryovial contains >5 x 10^5 cells in 1 ml freezing medium. Our EPCs were maintained in suspension culture.

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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 EPC

I. Preparation of EPC Growth Medium
We recommend the use of BioChain's EPC growth medium (Cat#Z7030073) for culturing our human CD133+ EPC.
1. Thaw EPC growth medium supplement (Cat#) in a water bath at room temperature.
2. Prepare EPC growth medium by adding the entire volume (50 ml) of EPC growth medium supplement (Cat#Z7030075) to the bottle (500 ml) of EPC basal medium (Cat#Z7030074). BioChain's EPC 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 EPC growth medium in a 37°C water bath.

II. Thawing frozen cells
1. Warm EPC 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 15 ml tube. Rinse the vial with 1 ml of fresh growth medium; and combine the rinse with the cells in 15 ml tube. Centrifuge at 400g for 6 minutes to precipitate the cells. Remove the supernatant. Add 8 ml of fresh EPC medium and put into a T25 flask.
4. Incubate the cells at 37°C with 5% CO_2 and 95% air in a humidified incubator. Change medium every 3 days.

III. Sub-culturing cells
1. Subculture the cells when they reach $5x10^5$ cells/ml.
2. Aseptically transfer the cell suspension to a 50 ml tube and centrifuge at 400 g for 6 minute to precipitate the cells.
3. Remove the supernatant. Add 16 ml of fresh EPC growth medium and separate into 2 T25 flasks.
4. Incubate the cells at 37°C with 5% CO_2 and 95% air in a humidified incubator. Change medium every 3 days.

Note: Due to the cells' characteristics, the cell growth rate is getting slower and slower, the CD 133 biomarker may be lost after 5 days growing. We recommend any experiments be performed within 3 day growing, especially for early stage biomarker. There are some Endothelial Cell Growth Supplements commercially available, those supplements can be added to the medium for better growth, but may make the cells differentiate into endothelial cells faster.

Related Products

EPC Growth Medium (Cat#Z7030073)
EPC Basal Medium (Cat#Z7030074)
EPC Growth Medium Supplement (Cat#Z7030075)
EPC Freezing Medium (Cat#Z7030076)

References

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4. Kawamoto A, Losordo DW. Endothelial progenitor cells for cardiovascular regeneration. Trends Cardiovasc Med. 2008 Jan; 18(1):33-7. Review.
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