High-yield/large-scale isolation of HUVECs in Endothelial Cell Growth Medium 2

PromoCell®

Application note

Most research on vascular endothelium uses human umbilical vein endothelial cells (HUVECs). First isolated and cultured in the 70s [1], HUVECs soon became the mainstay of cell research in many laboratories, being readily available, quite easy to culture, highly proliferative, and able to migrate and invade new tissues. Besides their suitability for studies on macromolecule transport, blood coagulation and fibrinolysis, HUVECs are the most commonly used cell type for in vitro studies on angiogenesis. To evaluate the influence of different substances on the endothelium, HUVECs can be used in a tube formation assay that was first described by Kubota et al., in 1988 [2]. Cells are plated onto a basement-membrane-like substrate on which they rapidly form capillary-like tubules. Scientists can then screen for various factors able to promote or inhibit angiogenesis. Read more in our AppNote Endothelial Cell Tube Formation Assay (Angiogenesis Assay). Endothelial cell migration and invasion are also key features of angiogenesis. The Transwell® migration assay, first introduced as the Boyden chamber assay, measures the ability of HUVECs to move along a cytokine gradient [3; 4]. Transmigration assays allow researchers to determine the number of cells migrating through a membrane from an upper compartment to a lower compartment where chemotactic agents are present. Read more in our AppNote Endothelial Cell Transmigration and Invasion Assay. PromoCell supplies both HUVECs and a wide spectrum of human endothelial cells (ECs) from large and microvascular vessels. Our optimized media also support endothelial cell culture, especially HUVEC isolation and culture.

Here we show a procedure for a high- yield isolation of primary HUVECs in PromoCell's Endothelial Cell Growth Medium 2 (ECGM2) and the comparison with a competitor medium (Figures 1 and 2).

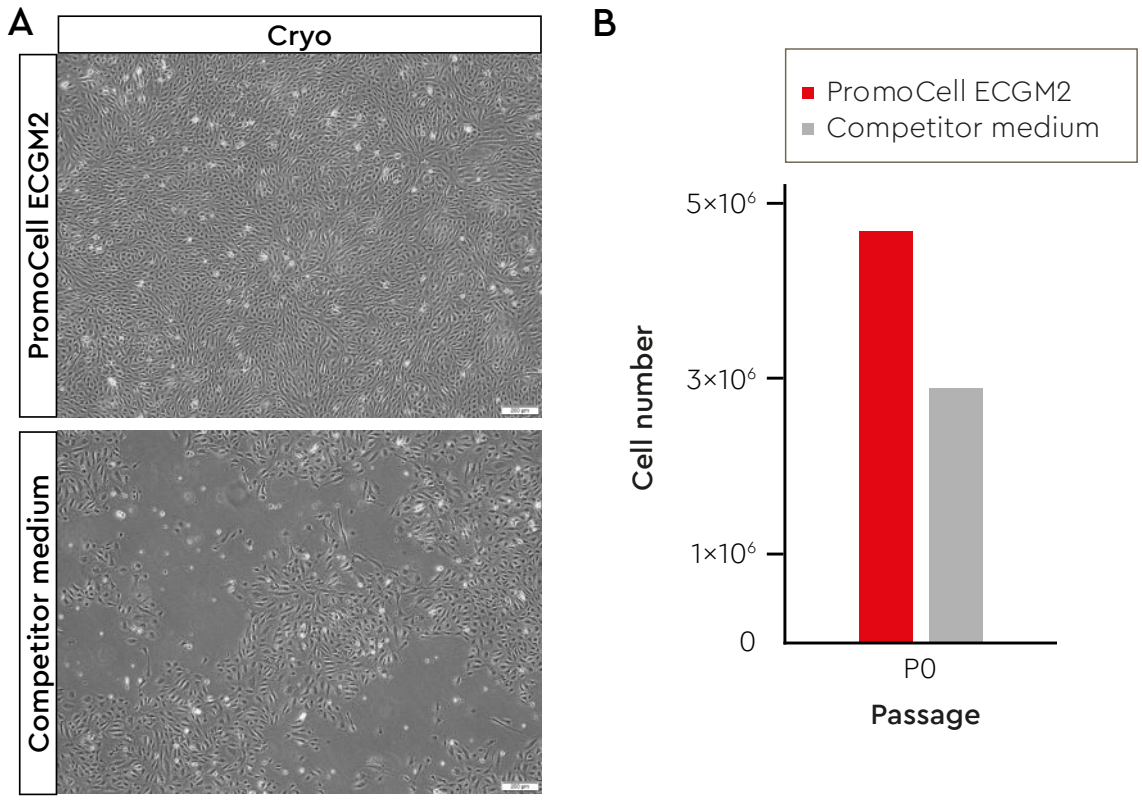

Fig. 1: HUVEC growth in PromoCell Endothelial Cell Growth Medium 2 (ECGM2) and competitor medium at P0. *Freshly isolated HUVECs were expanded in ECGM2 and a competitor medium and cryopreserved in P1. A, at the time of cryopreservation, cell density in the ECGM2 sample was higher than in the competitor medium. B, quantitative evaluation of cell number at the time of cryopreservation. There were 1.6x more cells in the ECGM2 sample than in the competitor medium.*

Background

The cells can be obtained from one sin- gle donor, three or four pooled donors, or donors that have been prescreened for VEGF response.

PromoCell Endothelial Cell Growth Media contain all of the growth factors and supplements that are necessary for optimal growth of human endothelial cells. Endothelial Cell Growth Medium 2 lacks the undefined Endothelial Cell Growth Supplement (ECGS, bovine hypothalamic extract), but contains Insulin-like Growth Factor (Long R3 IGF-1), and Vascular Endothelial Growth Factor. PromoCell Endothelial Cell Growth Media do not contain antibiotics or antimycotics and are formulated for use in an incubator with an atmosphere of 5% CO2.

HUVECs give scientists essential insights into the fascinating world of the vasculature. A better understanding of pathological changes within this vital infrastructure would bring scientists one step closer to developing significant therapeutic approaches for a range of diseases.

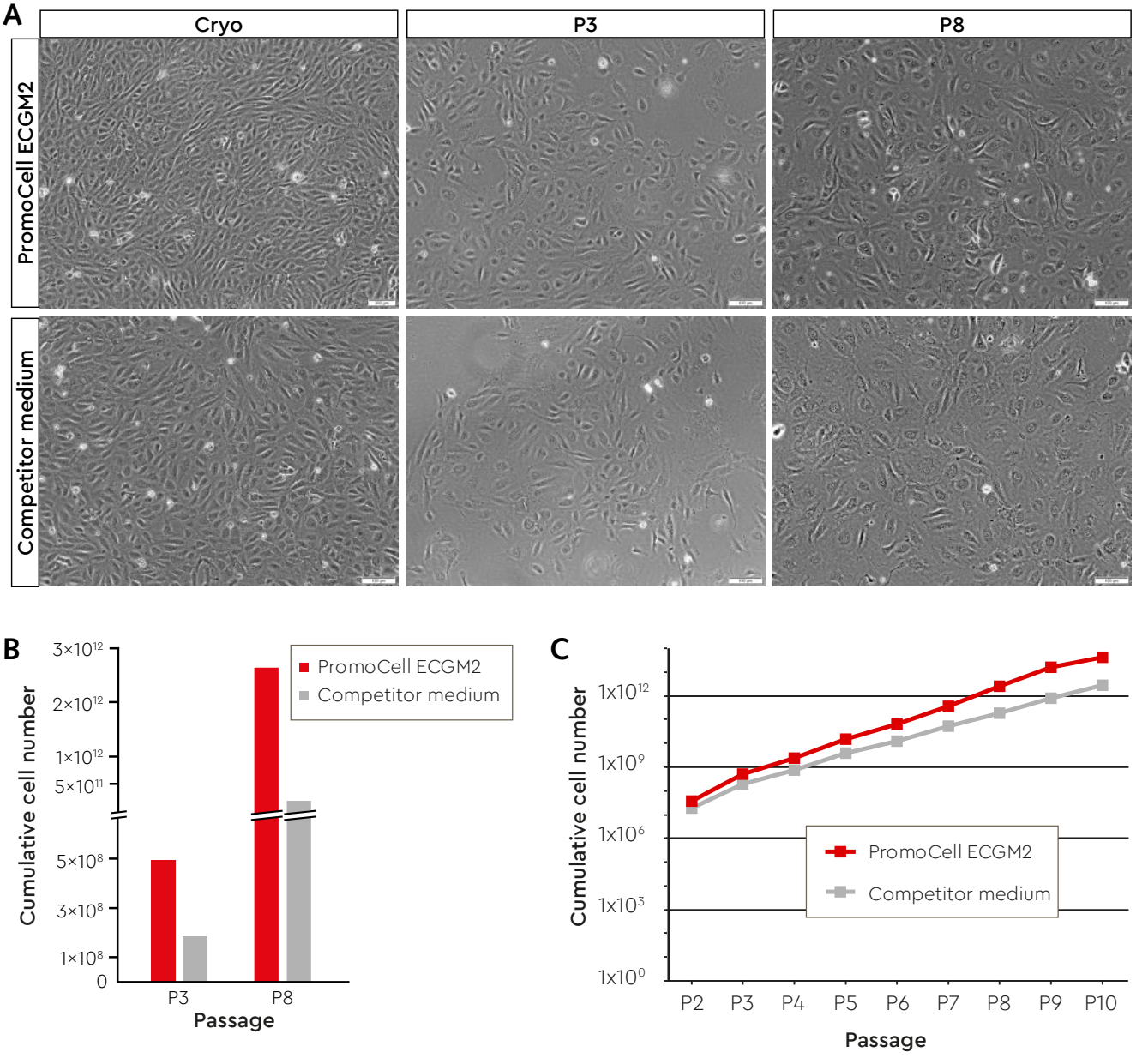

Fig. 2: Comparison of HUVEC growth in PromoCell's Endothelial Cell Growth Medium 2 (ECGM2) and competitor medium after thawing over 10 passages. *Cryopreserved HUVECs were thawed and plated with 5,000 cells/cm2 in ECGM2 or competitor medium on conventional plastic dishes. Subsequently, cells were cultured for 10 passages using a passage interval of 3–4 days.* ***A:*** *HUVEC morphology in cryopreservation, P3 and P8: the cells in ECGM2 are smaller and exhibit more intact cell edges. These differences were visible through all phases of the growth promotion test.* ***B:*** *cumulative cell number in P3 and P8 of the growth promotion test to indicate the huge difference in cell yields. C: cumulative cell numbers (logarithmic scaling) in ECGM2 are higher than in the competitor medium in all passages.*

HUVEC isolation protocol

I. HUVEC isolation procedure

Materials

- 70% EtOH
- Scalpel, syringe, canulae, centrifuge tube, cell culture vessels
- Physiological buffer (PBS, HepesBSS, HBSS or similar)
- Dispase solution
- PromoCell endothelium and Endothelial Cell Growth Medium 2 (C-22011)

Use aseptic techniques and a laminar flow bench.

1

Prepare Endothelial Cell Growth Medium 2

Thaw the SupplementMix or SupplementPack at 15 – 25°C. Aseptically mix the supplement solutions by carefully pipetting up and down. Then transfer the entire content of each supplement to the Basal Medium. Close the bottle and swirl gently until a homogenous mixture is formed.

Note: Light flocculation may be seen when thawing the supplements containing Heparin. This does not affect activity. Optionally, the precipitate can be removed by centrifugation under sterile conditions. After adding the supplements to the Basal Medium, the shelf life of the complete medium is 6 weeks at 4 – 8°C. Do not freeze the complete medium.

2

Adjust the medium to room temperature

Calculate the amount of PromoCell's Endothelial Cell Growth Medium 2 needed for isolation. Prewarm only an aliquot of the complete medium and keep the remaining medium refrigerated at 4 – 8°C.

3

Cut the ends of the umbilical cord with a sterile scalpel for clean access to the vein

Clean the umbilical cord using a paper towel rinsed with ethanol. Ensure that the umbilical cord is not damaged. Remove any damaged parts. Cut the ends of the umbilical cord with a sterile scalpel and locate the vein.

4

Insert a canula at one end of the vein and fix it with a vessel clamp

5

Rinse and perfuse umbilical vein with a physiological buffer

6

Insert a second canula on the other end and attach a vessel clamp to prevent leakage.

7

Fill the vein with Dispase solution using a syringe and incubate the umbilical cord at 37°C.

8

Carefully massage the umbilical cord to release the endothelial cells from the surrounding tissue

9

Rinse with physiological buffer

Perfuse the vein using a physiological buffer and collect the cell suspension in a sterile centrifuge tube.

10

Centrifuge the cells

Centrifuge the cells and resuspend the cell pellet in PromoCell's Endothelial Cell Growth Medium 2 and seed in TC vessels.

11

Change the medium every 2–3 days until the culture is subconfluent

Products

Media	Size	Catalog number
Endothelial Cell Growth Medium 2	500 ml	C-22011
Dulbecco's PBS, with or without Ca2+/Mg2+	500 ml	C-40230/C-40232
HEPES Buffered Saline Solution	30 ml/125 ml/250 ml	C-40000/C-40010/C-40020

Related products

Media	Size	Catalog number
Endothelial Cell Growth Medium	500 ml	C-22011
Endothelial Cell Growth Supplement/Heparin	2 ml	C-30120
Human Umbilical Vein Endothelial Cells 2 (HUVEC 2) single donor (isolated in Endothelial Cell Growth Medium 2)	500,000 cells	C-12206
Human Umbilical Vein Endothelial Cells 2 (HUVEC 2) pooled donor (isolated in Endothelial Cell Growth Medium 2)	500,000 cells	C-12208
Human Umbilical Vein Endothelial Cells (HUVEC) single donor	500,000 cells	C-12200
Human Umbilical Vein Endothelial Cells (HUVEC) pooled donor	500,000 cells	C-12203
DetachKit	3 × 30 ml	C-41200

References

1. Jaffe et al., Culture of human endothelial cells derived from umbilical veins. Identification by morphologic and immunologic criteria. J Clin Invest., 1973. 52(11):2745–56. doi: 10.1172/JCI107470
2. Kubota, Y., et al., Role of laminin and basement membrane in the morphological differentiation of human endothelial cells into capillary-like structures. J Cell Biol, 1988. 107(4):1589–98.
3. Boyden, S., The chemotactic effect of mixtures of antibody and antigen on polymorphonuclear leucocytes. J Exp Med, 1962. 115:453–66.
4. Chen, H.C., Boyden chamber assay. Methods Mol Biol, 2005. 294:15–22.

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