Endothelial Cell Transmigration and Invasion Assay

PromoCell

Application Note

Background

Angiogenesis is the physiological development of new blood vessels from existing ones, a process that is essential for embryonic and fetal development and organ growth, supports the healing of wounds and skeletal growth, and is also an integral part of pregnancy and the female reproductive cycle [1; 2]. It is triggered by tissue hypoxia or insufficient oxygen tension [3]. Newly developed blood vessels lined with endothelial cells supply oxygen and nutrients to tissues, promote immune surveillance by hematopoietic cells, and remove waste products [2].

Angiogenesis is a tightly regulated event that is balanced by pro- and antiangiogenic signals including integrins, chemokines, angiopoietins, oxygen sensing agents, junctional molecules and endogenous inhibitors [4]. It occurs in several well-characterized stages: At first, stimulatory signals bind to surface receptors on vascular endothelial cells. Activated endothelial cells then release proteases that degrade the underlying basement membrane and allow endothelial cells to migrate out of the existing blood vessel. The endothelial cells begin to proliferate and form sprouts that extend toward the source of the angiogenic stimulus.

While physiological angiogenesis is a highly organized sequence of cellular events, pathological angiogenesis is less well controlled, with vessels rarely maturing, remodeling or regressing in response to disease [5]. The development and delivery of anti-angiogenic drugs, especially in cancer therapy, is a large field of endeavor, and restoring vascular homeostasis holds great potential for the treatment of ischemic tissue diseases [1].

The key challenge in angiogenesis research is still selecting the right combination of *in vitro* and *in vivo* assays from the wide available range for obtaining meaningful and translatable results. In order to study angiogenesis in all its variety, a robust, rapid and reproducible assay with a reliable readout is needed.

Since endothelial cell migration and invasion are essential to angiogenesis, the so-called transwell migration assay and the cell invasion assay are helpful tools for studying the underlying mechanisms of angiogenic events. It is important to clearly distinguish between the terms "migration" and "invasion": "migration" can simply be defined as the process by which cells move from one location to the other. Usually, this is directed by extrinsic biochemical signals. The growth surface is not specified. Cells can migrate on 2D substrates like basement membrane extract, collagen fiber or a plastic culture scaffold [6]. Cell "invasion", on the other hand, is related to and encompasses cell migration. Invading cells do more than migrating cells. Invasion defines the ability of cells to move through a 3D matrix. It is a complex multistep process that involves adhesion, proteolysis of ECM components, reorganization of the microenvironment, and migration through the matrix [6]. According to this definition, migration is a prerequisite for invasion. Cells cannot invade a 3D matrix unless they are able to migrate. On a 2D substrate, however, it is possible for them to migrate without invading.
Both cell migration and cell invasion can be analyzed *in vitro* using the above-mentioned assays.

In Vitro Transmigration Assay

The transwell migration assay was originally introduced as the Boyden chamber assay for analyzing leucocyte chemotaxis [7; 8]. It used the migration of endothelial cells along a cytokine gradient (chemotaxis) to measure the chemotactic capability of test substances *in vitro*. It involves two medium-filled compartments separated by a microporous membrane. As a rule, cells are placed in the upper compartment and allowed to migrate through the pores of the membrane into the lower compartment, where chemotactic agents are present. After an appropriate incubation time, the membrane between the two compartments is fixed and the number of cells that have migrated to the lower side is determined.

In Vitro Invasion Assay

The cell invasion assay is closely related to the transwell migration assay, but answers a different set of questions. While the transwell migration assay measures the number of cells passing a porous membrane, the cell invasion assay focuses on invasive cell migration via an extracellular matrix. Invasive migration is a major process in angiogenesis but also plays a significant role in pathological events such as cancer development and metastasis [9]. The cell invasion assay analyzes the proteolytic activity of endothelial cells and their directed migration *in vitro*. It is possible to analyze both the influence of different substances on proteolytic activity and their chemotactic efficacy in a single assay. Like the transwell migration assay, the cell invasion assay involves two compartments separated by a membrane with a precisely defined pore size. The membrane is covered by a matrix that mimics the basement membrane of blood vessels. A potential chemotactic factor is placed

in one compartment and a gradient develops across the membrane. Endothelial cells introduced to the other compartment degrade the matrix and then migrate along this gradient. After a suitable incubation time, the cells that have migrated through the membrane can be counted after fixing the membrane and staining them [9]. A general workflow for performing the transmigration and invasion assay is shown in Fig. 1.

Substances that have already been analyzed using the transwell migration assay or the cell invasion assay are VEGF [10], FGF-1 [11], FGF-2 [12] and kinase inhibitors like SU5614 [13]. Because of their ease of use and the fact that they yield results quickly with a clear readout, both assays are still up to date in current laboratory practice. Recently, for example, they have been used to analyze the influence of renal endothelial cells in diabetic nephropathy [14], the role of retinal endothelium in ocular disease [15], the interplay between cancer cells and endothelial cells in pathological tumor invasiveness [16] and the influence of aquaporin activity on the invasiveness of colon cancer cells [17].

The transwell migration assay and the invasion assay have certain advantages over other frequently used assay types. They permit a certain degree of flexibility in performing migration or invasion experiments. For example, the effect of inhibitors that are specific to intracellular signaling molecules or that of functional antibodies blocking individual cell surface proteins can be assessed simply by adding the components to the upper chamber containing the cells. Both assays are fast and easy to set up and perform. The transwell migration assay in particular permits migration analysis independently of cell proliferation: cell migration through the membrane generally only takes a few hours (4-6 h). This is much faster than the time that cells need to complete their cycle.

Both assays can be used with either adherent or nonadherent cells [9], and they can be easily quantified using standard laboratory materials and apparatus such as solutions for fixing and staining cells and an inverted microscope. There is no need for costly or unusual equipment.

Workflow for Transmigration and Invasion Assay

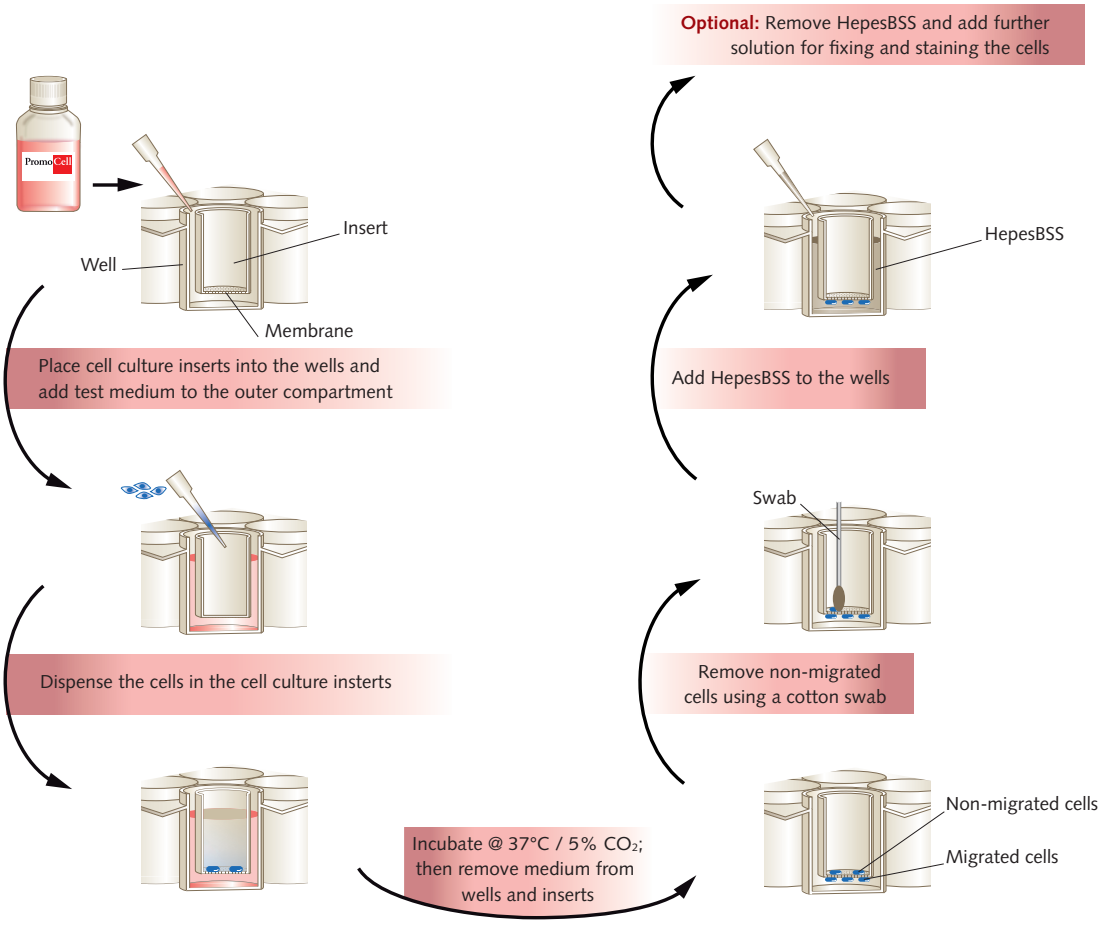

Fig. 1: Protocol overview showing the general steps for performing the Transmigration and Invasion Assay

Use aseptic techniques and a laminar flow bench.

A) Transmigration Assay

The transmigration assay allows to investigate the chemotactic ability of test substances *in vitro*. The assay is based on two compartments separated by a membrane with an accurately defined pore size. A potential chemotactic factor is placed in one compartment and a gradient develops across the membrane. Endothelial cells placed in the other compartment migrate along this gradient. Within several hours after adding the test substance, the number of cells that have migrated through the membrane can be counted.

Transmigration Assay Materials

I. Materials

- PromoCell Endothelial Cells (see page 8)
- PromoCell Endothelial Cell Growth Medium (see page 8)
- Cell Culture Dish (e.g. Corning, Product No. 353 0045)
- PromoCell DetachKit (C-41210), incl. HepesBSS, Trypsin/EDTA solution and trypsin neutralizing solution (TNS)
- 24-well flat-bottom cell culture plate (e.g. Corning, Product No. 353 226)
- 24-well cell culture inserts with 8 µm pore size and transparent PET membrane (e.g. Corning, Product No. 353 097)
- Assay medium: Endothelial Cell Basal Medium (C-22210) + 10% FCS
- Test substance with potential chemotactic ability
- As positive control hbFGF-2 (e.g. PromoKine, C-60240 or C-60242A) or hVEGF-165 (e.g. PromoKine, C-64420 or C-64420A) can be used.

Recommended:

- PromoKine Cell Migration/Chemotaxis Assay Kits (see page 10)
- Includes cell migration chamber, control migration inducer, cell dissociation solution, wash buffer and cell migration dye.

Transmigration Assay Protocol

II. Transmigration Assay Protocol

1. **Prepare a culture of endothelial cells**
   Plate PromoCell Endothelial Cells at 5.000 cells per cm^2 (or as recommended in the respective product manual) in a suitable culture vessel using the recommended endothelial growth medium (see page 8). Replace culture medium every 2-3 days. Allow the cells to reach 70-90% confluency.

2. **Prepare the assay medium**
   Prepare an appropriate amount of assay medium by adding 10% FCS to the Endothelial Cell Basal Medium (e.g. 9 ml basal medium + 1 ml FCS).

3. **Adjust media and reagents to room temperature**
   Pre-warm the assay medium and the components of the PromoCell DetachKit at room temperature for 1-2 hours.
   Hint: Place an appropriate amount of methanol (98%) at 4°C if you plan to fix and stain the cells immediately after the assay (see page 7: C) Fixation and Analysis of Migrated Cells).

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4. **Prepare the test medium**
   Dissolve the test substance in assay medium. 750 µl of the test medium should be used per well of the 24-well plate. We recommend to prepare one additional test medium with 20 ng/ml VEGF or bFGF to be used as a positive control. As negative control assay medium without any chemotactic factor can be used.
   Note: Do not add more than 250 µl of the test substance to the assay medium. If the test substance is dissolved in DMSO or alcohol, make sure that the final DMSO or alcohol concentration in the test medium is below 1% (v/v).

5. **Prepare 24-well plates and add test medium**
   Place cell culture inserts into the wells of the 24-well plate. Add 750 µl test medium to the outer compartment of each well.

6. **Detach endothelial cells**
   Endothelial cells should be 70-90% confluent. Remove medium from the culture vessel and wash the cells by adding 200 µl HepesBSS per cm^2 of vessel surface. Remove the HepesBSS and add 100 µl Trypsin/EDTA solution per cm^2 of vessel surface. Close the vessel and examine cells under a microscope. When the cells start to detach, gently tap the side of the vessel to loosen the remaining cells. Neutralize trypsin solution by adding 100 µl TNS per cm^2 of vessel surface and gently shake the culture vessel for 30 seconds.

7. **Count the cells**
   Transfer the cell suspension into a centrifuge tube and spin the tube at 220 g for 4 min at room temperature. Remove the supernatant and resuspend the cell pellet in 5 ml assay medium. Determine cell number according to your standard procedure.

8. **Seed the cells in the cell culture inserts**
   Dilute the cell concentration to 1x 10^5 cells/ml with assay medium. Carefully add 500 µl of the cell suspension (= 50.000 cells) into the cell culture inserts in the 24-well plate.

9. **Start transmigration experiment**
   Incubate cells in the 24-well plate inserts in a humidified incubator (37°C, 5% CO_2) for 3-6 hours. During the incubation time a gradient of the test substance develops across the membrane. Cells migrate through pores along the gradient to the bottom side of the membrane and adhere there.
   Note: The optimal incubation time depends on the type and quantity of the test substance. For instance, the optimal incubation time for VEGF (20 ng/ml) is 4 hours.

10. **Remove non-migrated cells on the upper side of the membrane**
    Carefully remove the medium from all cell culture inserts. Then, remove the inserts using tweezers and aspirate the medium from each well. After returning the inserts into the well, remove non-migrated cells on the upper side of the membrane using a cotton swab. Pipet HepesBSS into the gap between the well and the insert.
    Note: Handle membranes with extreme care and do not allow them to dry out.

11. **Proceed with fixation and staining of migrated cells**
    For fixation and staining of migrated cells proceed with the respective protocol listed in section C (page 7).

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B) Invasion Assay

This assay analyzes the proteolytic activity of endothelial cells and their directed migration *in vitro*. It is possible to analyze the influence of test substances on proteolytic activity and chemotactic efficacy in one assay. The assay uses a simplified Boyden Chamber-like design with two compartments separated by a membrane with an accurately defined pore size. The membrane is covered by a matrix which mimics the basement membrane of blood vessels. A potential chemotactic factor is placed in one compartment and a gradient develops across the membrane. Endothelial cells placed in the other compartment degrade the matrix and migrate along this gradient. Within 24 hours after adding the test substance, the number of cells that have migrated through the membrane can be counted.

Invasion Assay Materials

I. Materials

- PromoCell Endothelial Cells (see page 8)
- PromoCell Endothelial Cell Growth Medium (see page 8)
- Cell Culture Dish (e.g. Corning, Product No. 353 0045)
- PromoCell DetachKit (C-41210), incl. HepesBSS, Trypsin/EDTA solution and trypsin neutralizing solution (TNS)
- Corning BioCoat Matrigel Invasion Chambers (Corning, Product No. 354 480)
- Assay medium: Endothelial Cell Basal Medium (C-22210) + 10% FCS
- Test substance with potential chemotactic ability.
- As positive control hbFGF-2 (e.g. PromoKine, C-60240 or C-60242A) or hVEGF-165 (e.g. PromoKine, C-64420 or C-64420A) can be used.

Recommended:

- PromoKine Cell Invasion Assay Kits (see page 10)
- Includes cell invasion chamber, basement membrane (laminin, collagen I or collagen IV), control invasion inducer, cell dissociation solution, wash buffer and cell invasion dye

Invasion Assay Protocol

II. Invasion Assay Protocol

1. **Prepare a culture of endothelial cells**
   Plate PromoCell Endothelial Cells at 5.000 Cells per cm^2 in a suitable culture vessel using the recommended endothelial growth medium (see page 8). Replace culture medium every 2-3 days. Allow the cell to reach 70-90% confluency.

2. **Prepare invasion assay chambers and adjust media and reagents to appropriate temperatures**
   Prepare an appropriate amount of assay medium by adding 10% FCS to the Endothelial Cell Basal Medium (e.g. 9 ml basal medium + 1 ml FCS). Add 750 µl assay medium to each well and 500 µl to the Matrigel-coated inserts. Place the 24-well plate in a humidified incubator (37°C, 5% CO_2) for 1-2 hours.
   Place residual assay medium and the components of the PromoCell DetachKit at room temperature for 1-2 hours.
   Hint: Place an appropriate amount of methanol (98%) at 4°C if you plan to fix and stain the cells immediately after the assay (see page 7: C) Fixation and Analysis of Migrated Cells).

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3. **Prepare the test medium**
   Dissolve the test substance in assay medium. 750 µl of the test medium should be used per well of the 24-well plate. We recommend to prepare one additional test medium with 20 ng/ml VEGF or bFGF to be used as a positive control. As negative control assay medium without any chemotactic factor can be used.
   Note: Do not add more than 250 µl of the test substance to the assay medium. If the test substance is dissolved in DMSO or alcohol, make sure that the final DMSO or alcohol concentration in the test medium is below 1% (v/v).

4. **Add the test medium to the wells**
   Remove the Matrigel-coated inserts from the wells of the 24-well plate using tweezers and aspirate the assay medium from each well. After returning the inserts into the wells, pipet 750 µl test medium into the gap between the well and the insert.
   Note: Handle membranes with extreme care and do not allow them to dry out.

5. **Detach endothelial cells**
   Endothelial cells should be 70-90% confluent. Remove medium from the culture vessel and wash the cells by adding 200 µl HepesBSS per cm^2 of vessel surface. Remove the HepesBSS and add 100 µl Trypsin/EDTA per cm^2 of vessel surface. Close the vessel and examine cells under a microscope. When the cells start to detach, gently tap the side of the vessel to loosen the remaining cells. Neutralize trypsin solution by adding 100 µl TNS per cm^2 of vessel surface and gently shake the culture vessel for 30 seconds.

6. **Count the cells**
   Transfer the cell suspension into a centrifuge tube and spin the tube at 220 *g* for 4 min at room temperature. Remove the supernatant and resuspend the cell pellet in 5 ml assay medium. Determine cell number according to your standard procedure.

7. **Seed the cells in the Matrigel-coated cell culture inserts**
   Dilute the cell concentration to 1x 10^5 cells/ml with assay medium. Remove the assay medium from Matrigel-coated inserts without touching the Matrigel-coated surface. Carefully add 500 µl of the cell suspension (= 50.000 cells) into the cell culture inserts.

8. **Start invasion experiment**
   Incubate cells in the Matrigel-coated inserts in a humidified incubator (37°C, 5% CO_2) for 16-28 hours.
   During the incubation time a gradient of the test substance develops across the membrane. In case of a positive stimulation by the test substance cells degrade the matrix, migrate through pores along the gradient to the bottom side of the membrane and adhere there.
   Note: The optimal incubation time depends on the type and quantity of the test substance. For instance, the optimal incubation time for VEGF (20 ng/ml) is 24 hours.

9. **Remove non-migrated cells on the upper side of the membrane**
   Carefully remove the medium from all Matrigel-coated inserts. Then, remove the inserts using tweezers and aspirate the medium from each well. After returning the inserts into the well, remove non-migrated cells on the upper side of the membrane using a cotton swab. Pipet 750 µl HepesBSS into the gap between the well and the insert.
   Note: Handle membranes with extreme care and do not allow them to dry out.

10. **Proceed with Fixation and Staining of migrated cells (section C, page 7).**

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C) Fixation and Analysis of Migrated Cells

Fixation and Analysis

I. Materials

- Methanol (≥ 99,9%) (e.g. Roth, Cat. No. 4627.4)
- Crystal Violet solution: 0,1% Crystal Violet (e.g. Sigma, Product No. C3886) in distilled water (w/v); pH 4,5
- Distilled water

II. Fixation Protocol

1. **Fix the membranes and dry the inserts**
   Carefully remove the HepesBSS from the wells and add 750 µl cold methanol (4°C) to the wells. Incubate for 20 min at room temperature.
   Carefully remove the methanol and wait for 30 minutes. During this time the membrane will air dry. After drying, the membrane can be stored at 4°C and processed later.
   Note: To remove HepesBSS and methanol pick each cell culture insert with a tweezer and move it out of the well. Then, remove the corresponding solution. Put the cell culture insert back into the well and either fill in the new solution through the little gap between the well and the insert or let the membrane air dry.

2. **Stain the cells**
   Add 750 µl Crystal Violet to the wells and incubate for 20 minutes at room temperature.
   Note: Alternatively, the cells can also be stained with fluorescent dyes or by immunocytochemical staining.

3. **Wash the membrane**
   Carefully remove the staining solution and wash three times thoroughly with distilled water.

4. **Proceed with Analysis**

III. Analysis

After staining of migrated cells, the transmigration assay and the invasion assay can by quantified by the following methods:

1. **Visual quantification**
   Fill 750 µl distilled water in the wells of the 24-well plate and count the migrated cells on the lower site of the membrane using an inverted microscope (see Fig. 2).

2. **Colorimetric quantification with a microplate reader**
   Fill 750 µl 10% acetic acid in the wells and incubate for 30 seconds while carefully shaking the 24-well plate. The cells on the membrane will be lysed by the acetic acid and the Crystal Violet in the cells will be released. Remove the insert from the 24-well plate and read the optical density of the 10% acetic acid at 595 nm using a microplate reader. For quantitative analysis compare the data with data from a serial dilution series.

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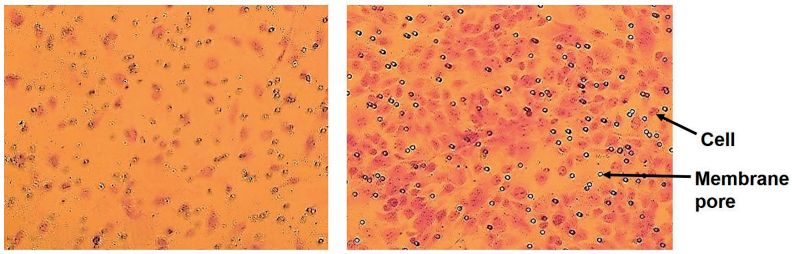

Fig. 2: Cells that have migrated through the membrane without VEGF (left) and with 20 ng/ml VEGF in the medium (right). Cells are stained with Crystal Violet.

Fixation and Analysis

Products

The following endothelial cell types have been successfully tested by PromoCell for *in vitro* transmigration and invasion assays:

Endothelial Cell Type	Size	Catalog Number
Human Umbilical Vein Endothelial Cells (HUVEC)	500.000 cryopreserved cells	C-12200
Human Dermal Microvascular Endothelial Cells (HDMEC), juvenile forskin	500.000 cryopreserved cells	C-12210
Human Dermal Microvascular Endothelial Cells (HDMEC), adult donor	500.000 cryopreserved cells	C-12212
Human Coronary Artery Endothelial Cells (HCAEC)	500.000 cryopreserved cells	C-12221
Human Aortic Endothelial Cells (HAoEC)	500.000 cryopreserved cells	C-12271
Human Pulmonary Microvascular Endothelial Cells (HPMEC)	500.000 cryopreserved cells	C-12281
Human Cardiac Microvascular Endothelial Cells (HCMEC)	500.000 cryopreserved cells	C-12285

Choice of Endothelial Cell Growth Medium

PromoCell Endothelial Cell Growth Media are available with and without ECGS (Endothelial Cell Growth Supplement, bovine hypothalamic extract) and VEGF (Vascular Endothelial Growth Factor) for large vessel (e.g. HUVEC) and microvascular endothelial cells (e.g. HDMEC). Both media variants are suitable for performing transmigration and invasion assays.
Endothelial Cell Growth Medium (MV) 2 lacks ECGS, but contains Insulin-like Growth Factor (Long R3 IGF) and VEGF. Generally, VEGF leads to higher endothelial cell proliferation in culture. But because of its multiple effects on cell metabolism, it may also interfere with certain experimental setups. In these cases, we recommend the use of Endothelial Cell Growth Medium which does not contain VEGF.

Endothelial Cell Origin	Growth Medium	Size	Catalog Number	Supplementation
Large Vessels	Endothelial Cell Growth Medium	500 ml	C-22110	Contains ECGS/Heparin*
	Endothelial Cell Growth Medium 2	500 ml	C-22011	Contains VEGF, IGF*
Microvascular Vessels, Coronary Artery, Aorta	Endothelial Cell Growth Medium MV	500 ml	C-22020	Contains ECGS/Heparin*
	Endothelial Cell Growth Medium MV 2	500 ml	C-22022	Contains VEGF, IGF, FGF*

* full supplementation details are available at www.promocell.com

Related Products

Cell Types and Media	Size	Catalog Number
Human Umbilical Vein Endothelial Cells (HUVEC) single donor	500,000 cryopreserved cells 500,000 proliferating cells	C-12200 C-12250
Human Umbilical Vein Endothelial Cells (HUVEC) pooled	500,000 cryopreserved cells 500,000 proliferating cells	C-12203 C-12253
Human Umbilical Vein Endothelial Cells (HUVEC) isolated in Growth Medium 2, single donor	500,000 cryopreserved cells 500,000 proliferating cells	C-12206 C-12207
Human Umbilical Vein Endothelial Cells (HUVEC) isolated in Growth Medium 2, pooled	500,000 cryopreserved cells 500,000 proliferating cells	C-12208 C-12209
Human Umbilical Vein Endothelial Cells (HUVEC) pre-screened	500,000 cryopreserved cells 500,000 proliferating cells	C-12205 C-12255
Human Umbilical Artery Endothelial Cells (HUAEC)	500,000 cryopreserved cells 500,000 proliferating cells	C-12202 C-12252
Human Aortic Endothelial Cells (HAoEC)	500,000 cryopreserved cells 500,000 proliferating cells	C-12271 C-12272
Human Coronary Artery Endothelial Cells (HCAEC)	500,000 cryopreserved cells 500,000 proliferating cells	C-12221 C-12222
Human Pulmonary Artery Endothelial Cells (HPAEC)	500,000 cryopreserved cells 500,000 proliferating cells	C-12241 C-12242
Human Saphenous Vein Endothelial Cells (HSaVEC)	500,000 cryopreserved cells 500,000 proliferating cells	C-12231 C-12232
Human Dermal Microvascular Endothelial Cells (HDMEC) juvenile foreskin	500,000 cryopreserved cells 500,000 proliferating cells	C-12210 C-12260
Human Dermal Microvascular Endothelial Cells (HDMEC) adult donor	500,000 cryopreserved cells 500,000 proliferating cells	C-12212 C-12262
Human Dermal Microvascular Endothelial Cells (HDMEC) pre-screened	500,000 cryopreserved cells 500,000 proliferating cells	C-12215 C-12265
Human Dermal Blood Endothelial Cells (HDBEC) juvenile foreskin	500,000 cryopreserved cells 500,000 proliferating cells	C-12211 C-12214
Human Dermal Blood Endothelial Cells (HDBEC) adult donor	500,000 cryopreserved cells 500,000 proliferating cells	C-12225 C-12226
Human Dermal Lymphatic Endothelial Cells (HDLEC) juvenile foreskin	500,000 cryopreserved cells 500,000 proliferating cells	C-12216 C-12218
Human Dermal Lymphatic Endothelial Cells (HDLEC) adult donor	500,000 cryopreserved cells 500,000 proliferating cells	C-12217 C-12219
Human Cardiac Microvascular Endothelial Cells (HCMEC)	500,000 cryopreserved cells 500,000 proliferating cells	C-12285 C-12286
Human Pulmonary Microvascular Endothelial Cells (HPMEC)	500,000 cryopreserved cells 500,000 proliferating cells	C-12281 C-12282
Human Uterine Microvascular Endothelial Cells (HUtMEC)	500,000 cryopreserved cells 500,000 proliferating cells	C-12295 C-12296
Human Pericytes from Placenta (hPC-PL)	500,000 cryopreserved cells 500,000 proliferating cells	C-12980 C-12981
Endothelial Cell Growth Medium (Ready-to-use)	500 ml	C-22010
Endothelial Cell Growth Medium 2 (Ready-to-use)	500 ml	C-22011
Endothelial Cell Growth Medium MV (Ready-to-use)	500ml	C-22020
Endothelial Cell Growth Medium MV 2 (Ready-to-use)	500ml	C-22022
Endothelial Cell Growth Medium Kit	500 ml	C-22110
Endothelial Cell Growth Medium 2 Kit	500 ml	C-22111
Endothelial Cell Growth Medium MV Kit	500ml	C-22120
Endothelial Cell Growth Medium MV 2 Kit	500ml	C-22121

Cell Types and Media	Size	Catalog Number
Endothelial Cell Basal Medium	500 ml	C-22210
Endothelial Cell Basal Medium, phenol red-free	500 ml	C-22215
Endothelial Cell Growth Medium SupplementMix	for 500 ml	C-39215
Endothelial Cell Growth Medium 2 SupplementMix	for 500 ml	C-39216
Endothelial Cell Growth Medium MV SupplementMix	for 500 ml	C-39225
Endothelial Cell Growth Medium MV 2 SupplementMix	for 500 ml	C-39226
Endothelial Cell Growth Medium SupplementPack	for 500 ml	C-39210
Endothelial Cell Growth Medium 2 SupplementPack	for 500 ml	C-39211
Endothelial Cell Growth Medium MV SupplementPack	for 500 ml	C-39220
Endothelial Cell Growth Medium MV 2 SupplementPack	for 500 ml	C-39221
DetachKit	30 ml 125 ml 250 ml	C-41200 C-41210 C-41220
Cryo-SFM	30 ml 125 ml	C-29910 C-29912

Cell Biology Products	Size	Catalog Number
Cell Invasion Assay Kit (BME, 8 µm, 24-well)	24 Assays	PK-CA577-K913
Cell Invasion Assay Kit (BME, 8 µm, 96-well)	100 Assays	PK-CA577-K912
Cell Invasion Assay Kit (laminin, 8 µm, 24-well)	24 Assays	PK-CA577-K915
Cell Invasion Assay Kit (laminin, 8 µm, 96-well)	100 Assays	PK-CA577-K914
Cell Invasion Assay Kit (collagen I, 8 µm, 24-well)	24 Assays	PK-CA577-K917
Cell Invasion Assay Kit (collagen I, 8 µm, 96-well)	100 Assays	PK-CA577-K916
Cell Invasion Assay Kit (collagen IV, 8 µm, 24-well)	24 Assays	PK-CA577-K919
Cell Invasion Assay Kit (collagen IV, 8 µm, 96-well)	100 Assays	PK-CA577-K918
Cell Migration/Chemotaxis Assay Kit (8 µm, 24-well)	12 Assays	PK-CA577-K909
Cell Migration/Chemotaxis Assay Kit (8 µm)	100 Assays	PK-CA577-K906
Cell Migration/Chemotaxis Assay Kit (5 µm, 24-well)	12 Assays	PK-CA577-K910
Cell Migration/Chemotaxis Assay Kit (5 µm, 96-well)	100 Assays	PK-CA577-K907
Cell Migration/Chemotaxis Assay Kit (3 µm, 24-well)	12 Assays	PK-CA577-K911
Cell Migration/Chemotaxis Assay Kit (3 µm, 96-well)	100 Assays	PK-CA577-K908
Angiogenesis Assay Kit	50 Assays	PK-CA577-K905
Calcein AM solution, 4 mM in DMSO	100 µl	PK-CA-707-80011-1
DAPI	10 mg	PK-CA707-40011
DAPI Solution (10 mg/ml)	10 mg	PK-CA707-40043
PromoFectin-HUVEC	0.1 ml 0.5 ml	PK-CT-2000-HUV-10 PK-CT-2000-HUV-50
More Cell Invasion Kits will be available soon at www.promocell.com/cell-biology.		

Growth Factors	Size	Catalog Number
FGF-2, human, recombinant (*E. coli*)	50 µg	C-60240
FGF-2, human, recombinant (HEK293)	10 µg	C-60242
FGF-2 (FGF-b, 146 aa), human, recombinant (Plant) - endotoxin-free	50 µg	C-60242A
FGF-1, human, recombinant (*E. coli*)	50 µg	C-60340
FGF-1, human, recombinant (Sf9)	10 µg	C-60343
VEGF-165, human, recombinant (*E. coli*)	10 µg	C-64420
VEGF-165, human, recombinant (HEK293)	10 µg	C-64423
VEGF-165, human, recombinant (Plant) - endotoxin-free	10 µg	C-64420A
VEGF-121, human, recombinant (*E. coli*)	10 µg	C-64410
VEGF-121, human, recombinant (Sf9)	10 µg	C-64409

More products and information are available at www.promocell.com/cell-biology.

References

[1] Van Hove, A.H. and D.S. Benoit, Depot-Based Delivery Systems for Pro-Angiogenic Peptides: A Review. Front Bioeng Biotechnol, 2015. 3: p. 102.

[2] DeCicco-Skinner, K.L., et al., Endothelial cell tube formation assay for the *in vitro* study of angiogenesis. J Vis Exp, 2014(91): p. e51312.

[3] Adams, R.H. and K. Alitalo, Molecular regulation of angiogenesis and lymphangiogenesis. Nat Rev Mol Cell Biol, 2007. 8(6): p. 464-78.

[4] Bouis, D., et al., A review on pro- and anti-angiogenic factors as targets of clinical intervention. Pharmacol Res, 2006. 53(2): p. 89-103.

[5] Staton, C.A., M.W. Reed, and N.J. Brown, A critical analysis of current *in vitro* and *in vivo* angiogenesis assays. Int J Exp Pathol, 2009. 90(3): p. 195-221.

6] Kramer, N., et al., *In vitro* cell migration and invasion assays. Mutat Res, 2013. 752(1): p. 10-24.

[7] Boyden, S., The chemotactic effect of mixtures of antibody and antigen on polymorphonuclear leucocytes. J Exp Med, 1962. 115: p. 453-66.

[8] Chen, H.C., Boyden chamber assay. Methods Mol Biol, 2005. 294: p. 15-22.

[9] Hulkower, K.I. and R.L. Herber, Cell migration and invasion assays as tools for drug discovery. Pharmaceutics, 2011. 3(1): p. 107-24.

[10] Poldervaart, M.T., et al., Prolonged presence of VEGF promotes vascularization in 3D bioprinted scaffolds with defined architecture. J Control Release, 2014. 184: p. 58-66.

[11] Mori, S., et al., A dominant-negative FGF1 mutant (the R50E mutant) suppresses tumorigenesis and angiogenesis. PLoS One, 2013. 8(2): p. e57927.

[12] Hussain, S., et al., Stilbene glycosides are natural product inhibitors of FGF-2-induced angiogenesis. BMC Cell Biol, 2009. 10: p. 30.

[13] Jia, H., et al., Vascular endothelial growth factor (VEGF)-D and VEGF-A differentially regulate KDR-mediated signaling and biological function in vascular endothelial cells. J Biol Chem, 2004. 279(34): p. 36148-57.

[14] Grutzmacher, C., et al., Aberrant production of extracellular matrix proteins and dysfunction in kidney endothelial cells with a short duration of diabetes. Am J Physiol Renal Physiol, 2013. 304(1): p. F19-30.

[15] Bharadwaj, A.S., et al., Role of the retinal vascular endothelial cell in ocular disease. Prog Retin Eye Res, 2013. 32: p. 102-80.

[16] Mierke, C.T., Cancer cells regulate biomechanical properties of human microvascular endothelial cells. J Biol Chem, 2011. 286(46): p. 40025-37.

[17] Dorward, H.S., et al., Pharmacological blockade of aquaporin-1 water channel by AqB013 restricts migration and invasiveness of colon cancer cells and prevents endothelial tube formation in vitro. J Exp Clin Cancer Res, 2016. 35: p. 36.

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