QCM™ Endothelial Cell Invasion Assay (24 well, colorimetric)

Catalog No. ECM210

Sufficient for analysis of 24 samples

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures.

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Introduction

Endothelial cells (EC) invade through the basement membrane (BM) to form sprouting vessels. The invasion process consists of the secretion of matrix metalloproteases (MMP) to degrade basement membrane, the activation of endothelial cells, and the migration of EC across the basement membrane. The understanding of EC invasion is important for studying the mechanism of angiogenesis in injured tissue as well as in disease such as cancer.

Cell migration may be evaluated through several different methods, the most widely accepted of which is the Boyden Chamber assay. The Boyden Chamber system uses two-chamber system which a porous membrane provides an interface between two chambers. Cells are seeded in the upper chamber and chemoattractants placed in the lower chamber. Cells in the upper chamber migrate toward the chemoattractants by passing through the porous membrane to the lower chamber. Migratory cells are then stained and quantified.

Millipore's QCM Endothelial Cell Invasion Assay provides an *in vitro* model to quickly screen factors that can regulate endothelial invasion. The assay is performed in an invasion chamber using a basement membrane protein coated on the porous insert. The level of coating and the pore size is optimized for endothelial cells so the researcher may utilize this kit to mimic physiological condition. After cell invasion has occurred, the researcher can select a staining method to quantify the number of cells that have invaded through the chamber. Millipore offers a colorimetric staining kit (ECM210) and a fluorometric kit staining reagent (ECM211) for both convenience and efficiency.

In addition to the Endothelial Cell Invasion Assay, Millipore offers:

- EndoGRO HUVEC (Cat. No. SCCE001)
- EndoGRO-LS complete Media Kit (Cat. No. SCME001)
- Tumor Necrosis Factor-α, recombinant human (Cat. No. GF023)
- QCM™ 3 µm Endothelial Cell Migration Assay - Fibronectin, Colorimetric (Cat. No. ECM200)
- QCM™ 3 µm Endothelial Cell Migration Assay - Fibronectin, Fluorometric (Cat. No. ECM201)
- QCM™ Leukocyte Transendothelial Migration Assay – Colorimetric (Cat. No. ECM557)
- QCM™ Tumor Cell Transendothelial Migration Assay – Colorimetric (Cat. No. ECM558)
- Endothelial Cell Characterization Kit (Cat. No. SCR023)
- *In Vitro* Vascular Permeability Assay (Cat. No. ECM640)
- *In Vitro* Angiogenesis Assay (Cat. No. ECM625)
- Fibrin *In Vitro* Angiogenesis Assay (Cat. No. ECM630)
- Alpha/Beta Integrin-Mediated Cell Adhesion Colorimetric Array Combo Kit (Cat. No. ECM532)
- Alpha/Beta Integrin-Mediated Cell Adhesion Array Fluorometric Combo Kit (Cat. No. ECM535)
- QCM™ 3µm 96-well Chemotaxis Cell Migration Assay (Cat. No. ECM515)

Kit Components

1. Cell Invasion Plate Assembly: (Part No. CS203020) Two 24-well plates each containing 12 ECMatrix™-coated 3 µm inserts per plate.
2. Cell Stain Solution: (Part No. 90144)* One bottle.
3. Extraction Buffer: (Part No. 90145) One bottle.
4. 24-well Stain Extraction Plate: (Part No. 2005871) One each.
5. 96-well Stain Quantitation Plate: (Part No. 2005870) One each.
6. Cotton Swabs: (Part No. 10202) Fifty each.
7. Forceps: (Part No. 10203) One each.

*Caution: Cell Stain Solution contains a small amount of crystal violet, which is toxic if swallowed or inhaled, and may cause irritation to the eyes, respiratory system, and skin. Handle with caution.

Materials Not Supplied

1. Precision pipettes: sufficient for aliquoting cells.
2. Harvesting buffer: EDTA or trypsin-based cell detachment buffer, or Millipore's non-mammalian cell detachment solution, Accutase™ (Cat. No. SCR005) as a gentle alternative.
3. Endothelial cells, for example: HUVECs cells (Cat. No. SCCE001)
4. Endothelium cell culture medium appropriate for subject cells, such as EGM-2 (Endothelial cell growth media-2)
5. Quenching Medium: serum-free medium, such as EBM-2 etc containing 5% BSA. Must contain divalent cations (Mg^{2+}, Ca^{2+}) sufficient for quenching EDTA in harvesting buffer.
6. Sterile PBS or HBSS to wash cells.
7. Distilled water
8. (Optional) Chemoattractant or pharmacological agent added to culture medium
9. Low speed centrifuge and tubes for cell harvesting.
10. CO_2 incubator appropriate for subject cells.
11. Hemocytometer or other means of counting cells.
12. Trypan blue or equivalent viability stain.
13. Microplate reader (540-570 nm detection) or spectrophotometer.
14. Sterile cell culture hood
15. (Optional) Graduated ocular (calibrated), or automated method for counting stained cells on a membrane.
16. Shaker

Storage

Store kit materials at 2-8℃ up to the expiration d ate provided on the kit label. Do not freeze.

Cell Harvesting

Prepare subject cells for investigation as desired. The following procedure is recommended for HUVECs and may be optimized to suit individual cell types.

1. HUVECs are maintained in EGM-2 (Endothelial cell growth media) containing serum and growth factors. We recommend using lower passage of HUVEC cells (passage 1-8). Pre-coat tissue cultureware with 0.1% gelatin. Grow cells until they reach 80% confluence. Wash cells once with PBS and serum-starve them in the basal media (without serum or growth supplements) containing 0.1 to 0.5 % BSA for 12-18 hrs.
2. Wash cells once with sterile Harvesting buffer (see Materials Not Supplied).
3. Add 3 mL Harvesting Buffer per 100 mm dish and incubate at 37℃ for 5-15 minutes.
4. Pipet cells off the dish gently and add to 10 mL Quenching Medium (see Materials Not Supplied) to inactivate trypsin/EDTA from Harvesting Buffer.
5. Centrifuge cells gently to pellet (1500 RPM, 5-10 minutes).
6. Gently resuspend the pellet in 10 mL Quenching Medium, depending upon the size of the pellet.
7. Count cells and bring to a volume that gives 1.0 x 10^6 cells per mL.

   For each 10 cm plate, final cell number is approximately 3 to 5 x10^5 cells.
8. If desired, add additional compounds (cytokines, pharmacological agents etc.) to cell suspension.

Assay Instructions

Perform the following steps in a tissue culture hood:

1. For optimal results, bring plates to room temperature (25℃) prior to initiating the assay.
2. Sterilize forceps with 70% ethanol and handle inserts with forceps.
3. Add 300 µL of prewarmed serum-free media to the interior of the inserts. Allow this to rehydrate the ECM layer for 15-30 minutes at room temperature.
4. After rehydration from step 3, carefully remove 250 µL of media from the inserts without disturbing the membrane
5. In a laminar flow hood, add 300 µL of serum-free media in the presence or absence of chemoattractant (e.g. EGM-2 with 2% fetal bovine serum) to the lower wells beneath the Boyden chambers.

   ***Note**: Air may get trapped at the interface. Ensure that the bottom of the Boyden chamber membrane contacts the media in the lower wells.*
6. Prepare a cell suspension containing 1.0 x 10^6 cells/mL according to cell harvesting instructions above.
7. Apply 100 to 200 µL of cell suspension on top of the insert.
8. Cover plates and incubate for 18 - 24 hours in a 37℃ in a CO_2 incubator.

Staining Procedure

The following steps may be performed in a non-sterile environment:

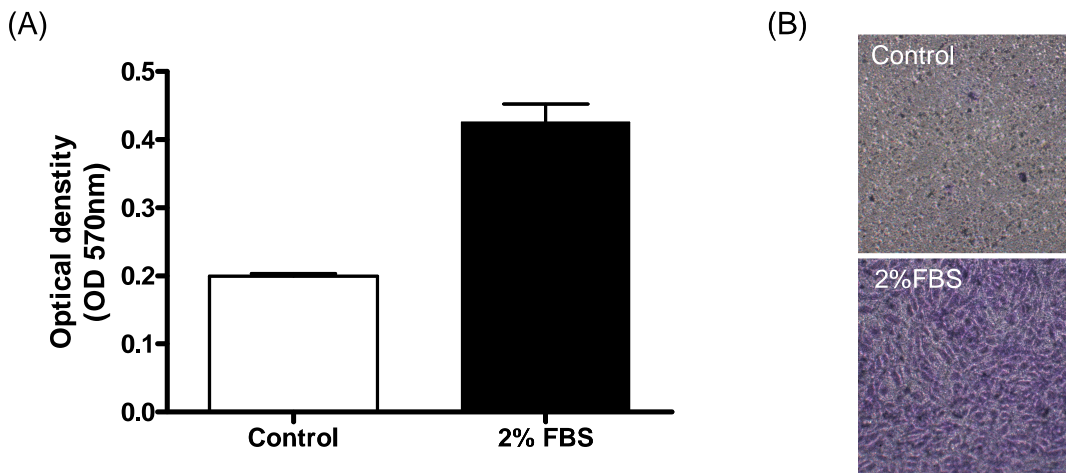
1. Prepare for staining by flattening the tips of 10-20 swabs and pressing the head of the swab firmly against a clean surface. These will be used to remove non-migratory cells from the top of the Boyden chambers.
2. Add 300 µL of the Cell Stain Solution (~6 drops) into the appropriate number of clean, empty wells within the Cell Invasion Plate Assembly.
3. Using a pipet, carefully remove the media from the upper chamber of the insert containing cells. Place each of these inserts into a clean well containing the Cell Stain Solution. Incubate for 10 to 20 minutes at room temperature.
4. Dip inserts into a beaker of water several times to rinse.
5. While the insert is still moist, use a cotton swab to remove any non-migratory cells from the interior of the insert. Carefully clean up all remaining cells to eliminate background stain. Repeat with new cotton swab if needed.

   Note: *Use care to not puncture the membrane. Thorough cleaning of non-migratory cells will significantly reduce the amount of background staining.*
6. Flick off excess water and allow to air dry.
7. Open and remove the included Stain Extraction Plate. Add 200 µL of Stain Extraction Solution to the required number of wells.
8. Transfer the chambers to the Stain Extraction Solution. Incubate for 5 – 10 minutes at room temperature on an orbital shaker.
9. Transfer 100 µL of the solution to the included 96-well Stain Quantitation Plate and read absorbance at 540- 570 nm.

Calculation of Results

Results of Millipore's QCM Endothelial Invasion Assay may be illustrated graphically. Running triplicates of each treatment is recommended to analyze the statistical significance of the outcome. A typical cell migration experiment should include a control chamber to assay invasion without chemoattractant. Cell invasion may be stimulated or inhibited in test wells through the addition of cytokines or other pharmacological agents.
The data below is for reference only and should not be used to interpret actual assay results.

Assay Example



(A) 150,000 HUVECs at passage 7 were seeded on top of Millipore Endothelial Invasion chambers. 300µL of 0.15% BSA in EBM-2 was applied as a control condition while 2% FBS in EBM-2 was applied to stimulate HUVEC invasion. Cells were allowed to invade through basal membrane for 24 hours at 37°C 5% CO_2 incubator before subject to staining. Result from triplicate samples with mean and standard error is shown. (B) Representative gross microscopic image of migratory HUVECs on the underside of a Boyden chamber, stained with Cell Stain Solution provided in the kit.

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