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

Catalog No. ECM211

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 to 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 invasion, researcher can choose the staining method to quantify the number of cells invade through the chamber. Millipore offers a colorimetric staining reagent kit (ECM210) and a fluorometric staining kit (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 3 µm 96-well Chemotaxis Cell Migration Assay (Cat. No. ECM515)

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Kit Components

ECM211-1

1. 24-well Cell Invasion Plate Assembly: (Part No. CS203020) Two 24-well plates each containing 12 ECMatrix™-coated 3 µm inserts per plate.
2. 24-well Stain Extraction Plate: (Part No. 2005871) One each.
3. 96-well Quantitation Plate: (Part No. CR201763) One each.
4. Forceps: (Part No. 10203) One each.

ECM211-2

1. Cell Detachment Solution: Accutase™ (Cat. No. SCR005A) One 100 mL bottle.
2. Calcein-AM: (Part No. CS202541) One 50 µg vial.

Materials Not Supplied

1. Precision pipettes: sufficient for aliquoting cells.
2. Harvesting buffer: EDTA or trypsin based cell detachment buffer, or other cell detachment formulations as optimized by individual investigators.
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. Starvation Medium: serum-free medium, such as EBM-2 etc containing 0.5 - 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 with Excitation/Emission of 495/520 nm or FITC channel
14. Sterile cell culture hood
15. (Optional) Graduated ocular (calibrated), or automated method for counting stained cells on a membrane.
16. Shaker

Storage

- ECM211-1 kit components should be stored at 2° to 8°C up to the expiration date provided on the kit. DO NOT FREEZE.
- ECM211-2 kit components must be stored at -20°C up to the expiration date provided on the kit.

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 the cells 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 3 mL sterile Accutase solution.
3. Add 5 mL Accutase solution per 100 mm dish and incubate at 37°C for 5-15 minutes. (Alternatively, trypsin, EDTA or other cell detachment formulations can be used as optimized by individual investigators.)
4. Pipet cells off the dish gently and rinse the plate with an additional 5 mL Accutase solution to collect residual cells. Combine both cell suspensions in one 15 mL conical tube.
5. Centrifuge cells gently to pellet (1500 RPM, 5-10 minutes).
6. Gently resuspend pellet in 10 mL Starvation Medium (see Materials not Supplied), depending upon the size of the pellet.
7. Count cells and bring to a volume that gives 1 to 2 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 cell culture hood:

1. For optimal results, bring plates to room temperature (25°C) prior to initiating 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 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 at 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°C in a CO_2 incubator.

Staining Procedure

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

1. Prepare Calcein-AM stock solution: Dissolve one vial of 50 µg Calcein-AM with 10 µL DMSO. Vortex vigorously and follow with brief centrifugation. Keep at room temperature until use.

   Note: *Calcein-AM should be kept in low moisture environment to prevent spontaneous hydrolysis, therefore, prepare Calcein-AM solution immediately before staining. Long-term storage of Calcein-AM should be kept at -20°C with minimum freeze/thaw cycles.*

2. Dilute Calcein-AM stock solution 1:5000 in starvation medium. For 24 samples, add 2 µL Calcein-AM stock solution to 10 mL of Starvation Medium. Aliquot 300 mL into the appropriate number of clean, empty wells within Rows C and D of the Cell Invasion Plate Assembly. Using the forceps, transfer the inserts from top rows to the bottom rows. Incubate at 37°C for 15 minutes.

   Note*: Removing excess Calcein-AM is optional; however, excessive Calcein-AM can lead to higher background. It is recommended to use one insert as a control containing no cells to subtract background for final data analysis.*

3. Open and remove the Stain Extraction Plate. Warm the Accutase (detachment solution) to 37°C and add 200 µL to each of the appropriate number of wells.

   Note*: Warming the detachment solution ensures maximum cell recovery from the insert.*

4. Transfer each stained insert to the Stain Extraction Plate. Incubate at 37°C for 30 minutes.

   Note*: If necessary, extend the incubation time to 60 minutes for cells difficult to dissociate.*

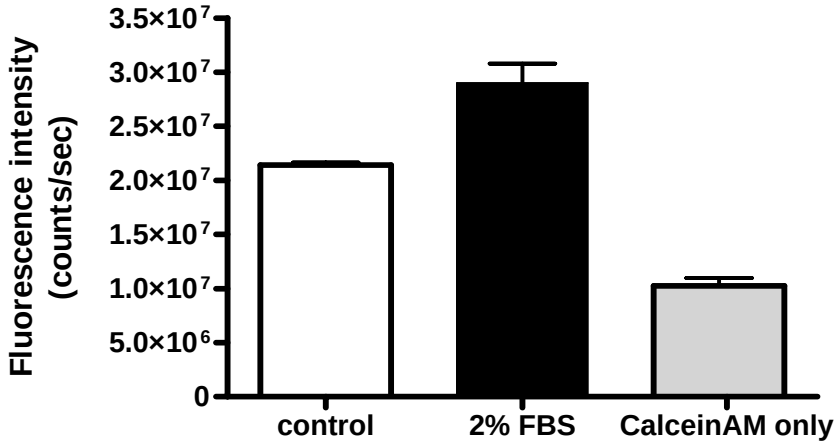
5. Transfer 100 to 200 µL of detached cell suspension to the included 96-well microtiter plate.

6. Determine the fluorescence intensity at 517 nm on a spectrophotometer. Alternatively, stained cells can be monitored via FITC channel.

Calculation of Results

Results of the QCM Endothelial Invasion Assay may be illustrated graphically. Performing 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



150,000 HUVECs, at passage 6, were seeded on top of Millipore Endothelial Invasion chambers. 300 µL EBM-2 with 0.15% BSA was applied as a negative chemoattractant control condition while 2% FBS in EBM-2 was applied as a chemoattractant to stimulate HUVEC invasion. Cells were allowed to invade through basal membrane for 24 hours at 37°C in a 5% CO_2 incubator before being subjected to staining. Results show triplicate sample data with means and standard errors.

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