QCM™ Endothelial Cell Migration Assay (96-well, fluorometric)

Catalog No. ECM202

Sufficient for analysis of 96 samples

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures.

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Introduction

Angiogenesis is a fundamental process involving the growth of new blood vessels from pre-existing vessels. It is important in development and wound healing, as well as diseases such as diabetic retinopathy and cancer. During angiogenesis, endothelial cells migrate from existing blood vessels to new tissue areas, proliferate, and assemble into new capillaries. The migration of endothelial cells is regulated by many angiogenic and anti-angiogenic factors, so it is critical for researchers to understand these mechanisms.

Millipore's QCM Endothelial Cell Migration Assay provides a quick and efficient system to study a compound's ability to induce or inhibit endothelial cell migration. This assay also allows for the screening of pharmacological agents, the evaluation of integrins or other adhesion receptors responsible for endothelial cell migration, the analysis of gene function in transfected cells, and for the determination of ECM protein involvement in cell movement.

Cell migration may be evaluated using several different methods; the most widely accepted being the Boyden Chamber assay. The Boyden Chamber system uses a two-chamber plate model in 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 stained and quantified.

Millipore's QCM Endothelial Cell Migration Assay measures endothelial cell movement towards chemoattractants. The bottom of the insert is coated with fibronectin, providing optimal conditions for endothelial cell adhesion and migration. The pre-coated Boyden chambers reduce assay time up to 80% by eliminating the overnight coating step required in traditional assays. Additionally, the assay does not require a high tech video system to determine motility changes. The 96 well format is suitable for high throughput screening.

In addition to the Endothelial Cell Migration 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 Endothelial Cell Migration Assay - Colorimetric (Cat. No. ECM200)
- QCM Endothelial Cell Migration Assay - Fluorometric (Cat. No. ECM201)
- QCM Endothelial Cell Invasion Assay, 24-well, Fluorometric (Cat. No. ECM211)
- 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)
- QCM 3µm 96-well Chemotaxis Cell Migration Assay (Cat. No. ECM515)
- Endothelial Cell Adhesion Assay Kit (Cat. No. ECM645)

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Application

Millipore's QCM Endothelial Cell Migration Assay is ideal for the study of endothelial cell migration in response to an angiogenic stimulus. The quantitative nature of the assay is especially useful for screening of pharmacologic agents. Boyden chambers containing 3 µm pore size membranes are ideal for the migration of endothelial cells such as HUVEC, but not optimal to measure fibroblast migration. Fibronectin protein is coated on the bottom of each chamber's porous membrane to provide optimal conditions for endothelial cell adhesion. BSA-coated control chambers provide an appropriate migration control. Each kit provides sufficient materials for the evaluation of 96 samples.

Kit Components

ECM202-1

1. Cell Migration Plate Assembly – Fibronectin-coated, 3 µm, 96-well Plate: (Part No. CS202539): One 96-well plate assembly with 3 µm pore inserts (Area = 0.3 cm^2) coated with human fibronectin; includes one 96-well feeder tray.
2. Cell Migration Plate Assembly – BSA-coated, 3 µm, 96-well Plate: (Part No. CS202538) One 96-well plate assembly of 3 µm pore inserts (Area = 0.3 cm^2) coated with BSA; includes one 96-well feeder tray.
3. 96 well Stain Quantitation Plate: (Part No. 2005870) Two each.
4. 96-well Cell Culture Tray: (Part No. 90129) Two 96-well feeder trays.

ECM202-2

1. Accutase™: (Cat. No. SCR005A) One 100 mL bottle of cell detachment solution.
2. Cytochalasin-D: (Part No. CS203023) One 15 µL vial at 5 mg/mL.
3. Calcein-AM: (Part No. CS202541) One 50 µg vial.

Storage

- ECM202-1 kit components should be stored at 2° to 8°C up to the expiration date provided on the kit. DO NOT FREEZE.
- ECM202-2 kit components must be stored at -20°C up to the expiration date provided on the kit.

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 (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

Cell Harvesting

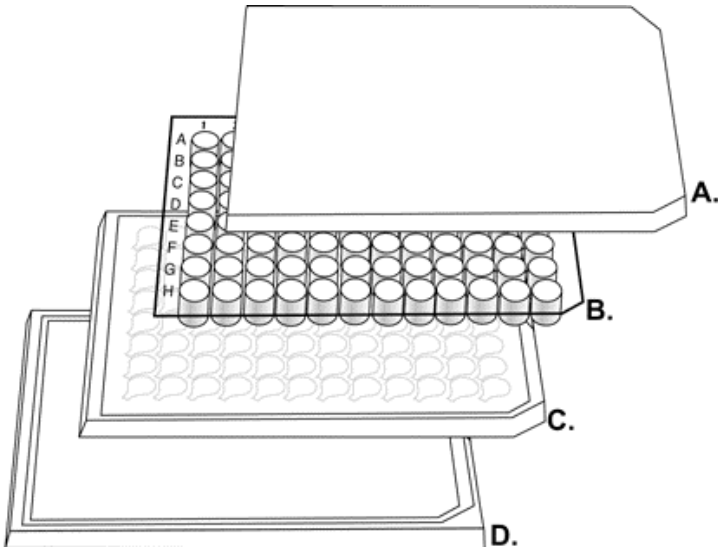
Prepare subject cells for investigation as desired. The following procedure is recommended for HUVEC cells and may be optimized to suit individual cell types.

1. HUVEC cells 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). Precoat tissue cultureware with 0.1% gelatin is recommended. Grow cells till they reach 80% confluence. Wash the cell 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 in 3 mL sterile Accutase solution.
3. Add 5 mLs of 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 5mL 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 the pellet in 10 mL starvation medium (EBM-2 with 0.1 to 0.5% BSA), depending upon the size of the pellet.
7. Count cells and bring to a volume that gives 1 x 10^5 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:



Cell Migration Plate Assembly
A. Lid
B. Cell Migration Chamber Plate
C. 96-well Feeder Tray
D. Base

1. For optimal results, bring plates and reagents to room temperature (25°C) prior to initiating assay.

   Note: For each condition tested, we recommend at least three replicates to avoid handling error. For negative controls, we recommend using serum-free media with 0.1 to 0.5% BSA.

2. Remove the lid (**A**) of the cell migration chamber plate (**B**) and to the wells of the feeder tray (**C**), add 150µL of serum-free media in the presence or absence of chemoattractant. See Step 1 image.

   Note: Avoid generating bubbles during pipeting or overfilling the well. The former will prevent air trapped in the interface and the latter prevents cross contamination.

3. (Optional) Cytochalasin-D, an actin polymerization inhibitor, is included in the kit as a negative control. Migration toward a chemoattractant is effectively abolished when cells are treated with 5 µM cytochalasin-D or in the presence of cytochalasin-D in the feeder tray.

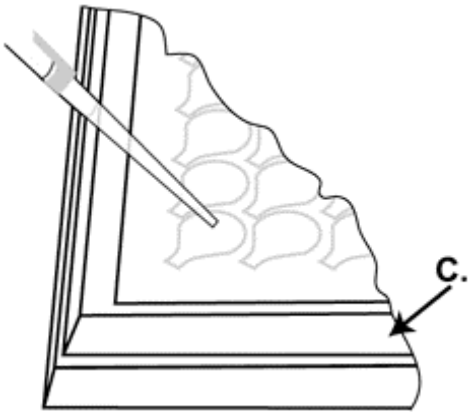
4. Prepare a cell suspension of 1 to 2 x 10^5 cells/mL, according to Cell Harvesting instructions above.

5. Insert the cell migration chamber plate (**B**) at a 45° angle from the bottom row and press it down gently but firmly all the way to the top row.

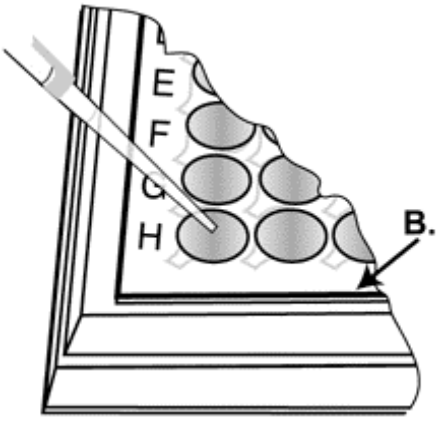
6. Add 100 µL cell suspension (10 to 20 x 10^3 cells) to each well. See Step 2 image.

7. Cover plate and incubate for 18 to 24 hours in a 37°C CO_2 incubator.

Step 1



Step 2



Staining Procedure

The following steps may be performed in a non-sterile environment on the second day of the assay.

1. Warm the Accutase cell detachment solution in a 37°C incubator.
2. Prepare the Calcein-AM stock solution by dissolving 50 µg Calcein-AM (one vial) with 10 µL DMSO. Vortex vigorously and follow with brief centrifugation. Keep at room temperature until use.

   Note: *Calcein-AM should be kept in a low moisture environment to prevent spontaneous hydrolysis. Therefore, prepare the Calcein-AM solution just prior to staining. For long term storage of Calcein-AM, store at -20°C with minimum freeze/thaw cycles.*
3. Dilute the Calcein-AM stock solution 1:100 using a basal medium (EBM-2 + 0.1 to 0.5% BSA). To analyze the entire plate, add 10 µL of the Calcein-AM stock solution to 1 mL basal medium. Add 5 µL to each well of the cell migration chamber plate (**B**). 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 three wells with no cells as a control to subtract background for final data analysis (See Assay Example, Figure (A)).*
4. Open and remove the 96-well Cell Culture Tray. Add 150 µL pre-warmed cell detachment solution to each sample well.
5. Once staining is complete, remove the cell migration chamber plate (**B**) and gently discard cells/media from the top side of the insert by flipping out the remaining cell suspension. Insert this plate into the 96-well Cell Culture Tray prepared in step 4. Incubate at 37°C for 30 minutes.

   Note*: Warming the cell detachment solution ensures maximum cell recovery from insert. If desired, extend the incubation time to 60 minutes for cells that are typically difficult to dissociate.*
6. Once incubation is complete, remove the cell migration chamber plate. Transfer 100 µL of the detached cell suspension to the included 96-well microtiter plate.
7. Determine the fluorescence intensity at 517 nm on a spectrophotometer. Alternatively, the stained cells can be monitored via the FITC channel.

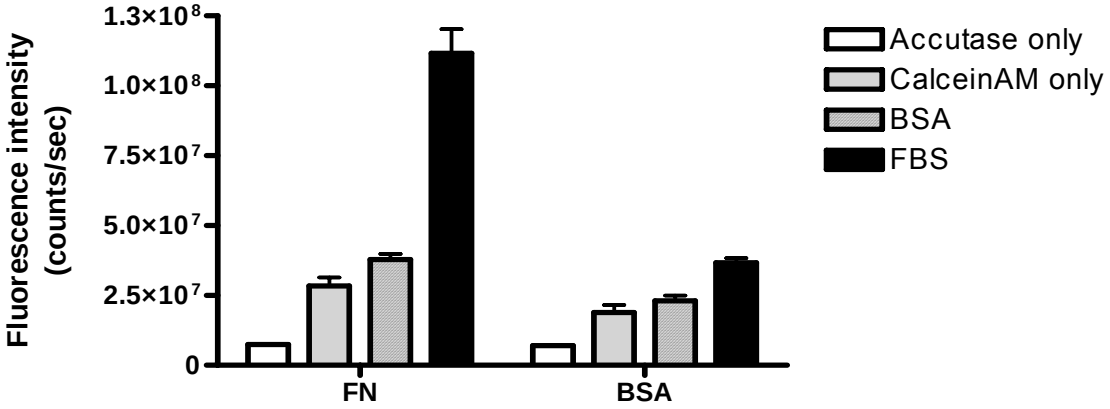
Calculation of Results

Results of the QCM Endothelial Migration Assay may be illustrated graphically. Performing triplicates of each treatment is recommended to analyze the statistic significance of the outcome. A typical cell migration experiment should include negative controls; chambers to assay cell migration without the presence of chemoattractant. Cell migration 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.

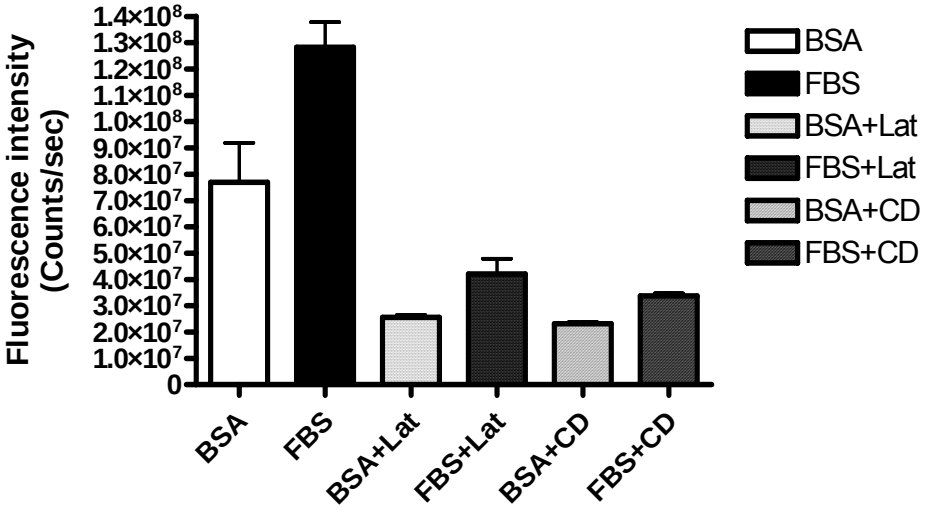
Assay Example

(A)



15,000 HUVECs at passage 7 were seeded into either a fibronectin (FN)-coated or a BSA-coated well within a cell migration chamber plate. 0.15% BSA in EBM-2 was applied as a negative control chemoattractant while 2% FBS in EBM-2 was applied as a chemoattractant to stimulate HUVEC migration. Cells were allowed to migrate for 24 hours at 37°C in a 5% CO_2 incubator before staining following Assays Instructions.

(B)



HUVECs at passage 6 were pretreated with inhibitor 2.3 µM latruculin A (Lat) or 5 µM cytochalasin D (CD) for 10 minutes. 20,000 cells were then seeded into either a fibronectin (FN)-coated or BSA-coated well within a cell migration chamber plate containing the same concentration of inhibitor in the feeder tray. Cells were allowed to migrate 24 hours at 37°C in a 5% CO_2 incubator before staining following Assay Instructions. Results show triplicate sample data with means and standard errors.

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