MILLIPORE

QCM™ 3 μm Endothelial Cell Migration Assay – Fibronectin, Colorimetric

Cat. No. ECM200

Sufficient for analysis of 12 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 pathologic diseases such as diabetic retinopathy and cancer. During angiogenesis, endothelial cells need to move out of existing vessels, migrate into new areas, proliferate and assemble into new capillaries. The migration of endothelial cells is regulated by many angiogenic factors and anti-angiogenic factors. It is critical for researchers to understand the mechanisms of endothelial cell migration.

Millipore 3µm QCM™ Endothelial Cell Migration Assay – Fibronectin, Colorimetric provides a quick and efficient system to study the ability of compounds to induce or inhibit endothelial cell migration. This assay also allows screening of pharmacological agents, evaluation of integrins or other adhesion receptors responsible for endothelial cell migration, analysis of gene function in transfected cells, and determination of ECM protein involvement in cell movement.

This versatile assay permits counting of individual migratory cells, and, more importantly, allows quantitative analysis by optical density (OD) using a standard microplate reader. This convenient assay allows large scale screening and quantitative comparison of multiple samples and includes individual migration controls for each sample.

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 were seeded in the upper chamber with a porous membrane at the bottom. Chemoattractants or test reagents were placed in the lower chamber. Cells in the upper chamber migrate toward to chemoattractants by passing the porous membrane and moving to the lower chamber. Migratory cells are then stained and counted.

In a standard Boyden assay, the pore diameter of the membrane is typically 3 to 12 µm, and is selected to suit the subject cells. Smaller pore size results in a greater challenge for the migrating cell. Most cells range in size from 30-50 µm and can migrate efficiently through 3-12 µm pores, whereas, lymphocytes (10 µm) can migrate through pores as small as 0.3 µm. Millipore's QCM™ 3µm Endothelial Cell Migration Assay – Fibronectin, Colorimetric utilizes a 3 µm pore size, as this pore size supports optimal migration for endothelial cells. The 3 µm pore size is not appropriate for fibroblast migration experiments.

The Boyden chamber design may be adapted to study different types of cell migration, including haptotaxis, random migration, chemokinesis and chemotaxis.

The Millipore QCM™ 3µm Endothelial Cell Migration Assay – Fibronectin, Colorimetric measures endothelial cell movement towards chemoattractants. The bottom of the insert is coated with fibronectin, which provide an optimal condition for endothelial cell migration and adhesion.

The QCM™ pre-coated Boyden chambers may reduce assay time by up to 80% by eliminating the overnight coating step required in traditional assays. This assay also does not require high tech video systems to determine motility changes.

In addition to time savings provided by pre-coating of migration chambers, the assay allows quantitative analysis of cell migration. After migration for the indicated time period, cells are stained and quantitated. In a departure from traditional Boyden methodology, stain is eluted with extraction buffer, transferred to a microplate, and measured spectrophotometrically. (Prior to elution, the user also has the option of counting cells individually, if desired.) Spectrophotometric absorbances correlate with cell migration. BSA-coated chambers serve as negative control "blanks" for each sample.

Each assay provides sufficient test and control chambers for the evaluation of 12 samples.

In addition to the QCM™ assay system, Millipore offers:

- ECM Cell Adhesion Arrays which contain strips with individual wells, each coated with one of seven ECM proteins (Cat. Nos. ECM540 and ECM545).
- CytoMatrix™ Cell Adhesion strips coated with one ECM protein: fibronectin, laminin, vitronectin, collagen I, or collagen IV (Cat. Nos. ECM101 to ECM105) or a combo pack of all five plates (Cat. No. ECM205).
- QuantiMatrix™ ECM protein ELISA kits for the measurement of soluble fibronectin or laminin (Cat. Nos. ECM300 and ECM310).

Application

The Millipore QCM™ 3µm Endothelial Cell Migration Assay – Fibronectin, Colorimetric is ideal for the study of endothelial cell migration in response to an angiogenic stimulus. The quantitative nature of this assay is especially useful for large scale screening of pharmacologic agents. BSA-coated control chambers provide an appropriate migration control. The 3 µm pore size in this assay is optimal for endothelial cells such as HUVEC, but not sufficient for fibroblast migration. The Millipore QCM™ 3µm Endothelial Cell Migration Assay – Fibronectin, Colorimetric assay is intended for research use only; not for diagnostic applications.

Each kit provides sufficient materials for the evaluation of 12 samples.

Kit Components

1. Fibronectin Test Plate: (Part No. CS201816) One 24-well culture plate, containing 12 human FN-coated Boyden chambers, sufficient for the evaluation of 12 test samples.
2. BSA Control Plate: (Part No. CS201815) One 24-well culture plate, containing 12 BSA-coated Boyden chambers, sufficient for the evaluation of 12 controls.
3. Cell Stain Solution*: (Part No. 20294) One vial – 10 mL.
4. Extraction Buffer: (Part No. 20295) One vial – 10 mL.
5. 24 well Stain Extraction Plate: (Part No. 2005871). One plate.
6. 96 well Stain Quantitation Plate: (Part No. 2005870). One plate.
7. Swabs: (Part No. 10202) 50 each.
8. Forceps: (Part No. 10203) 1 pair.

***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.*

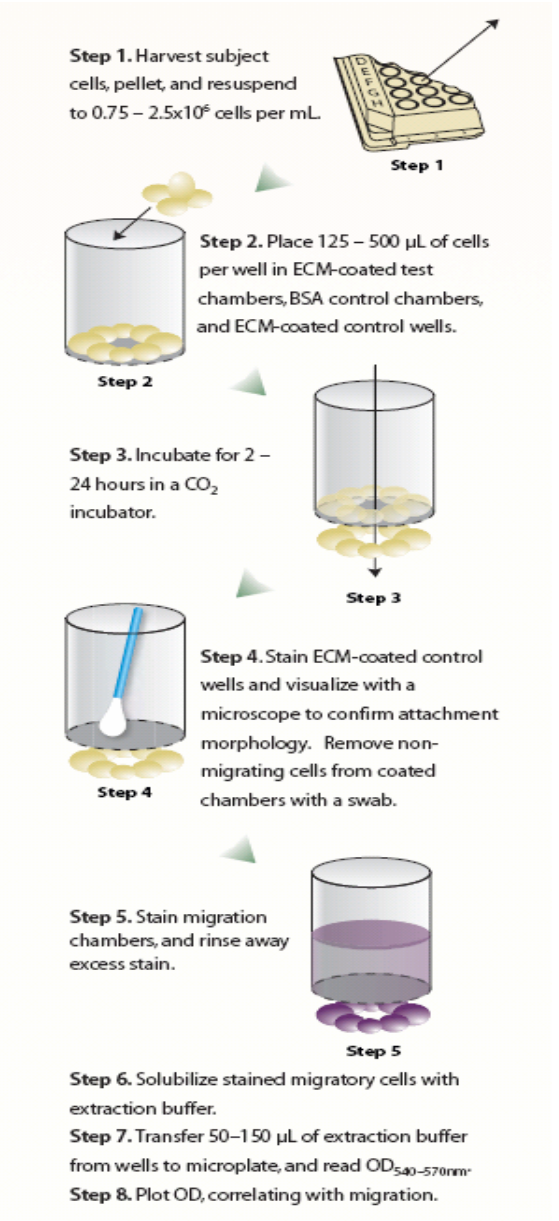
Materials Not Supplied

1. Precision pipettes: sufficient for aliquoting cells.
2. Harvesting buffer: EDTA or trypsin based cell detachment buffer, or other cell detachment formulations as optimized by individual investigators. Millipore's ready-to-use non-mammalian detachment solution, Accutase™ (Cat. No. SCR005) can also be used.
3. Endothelial cells (for example: HUVEC cells).
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 DMEM, MEM 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

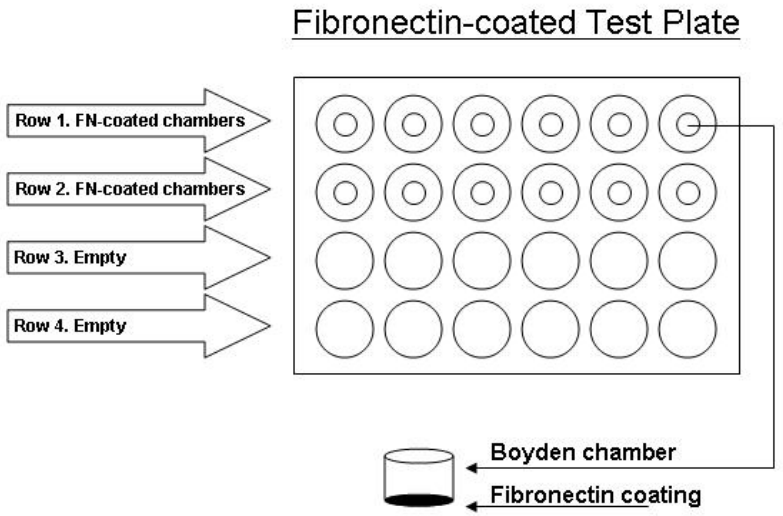
Assay Principle

The Millipore QCM™ 3µm Endothelial Cell Migration Assay – Fibronectin, Colorimetric utilizes Fibronectin-coated Boyden chambers and BSA-coated migration control Boyden chambers to provide a quantitative indication of cell migration. Cells migrating through 3 µm chamber pores are stained and counted or eluted with extraction solution. Optical density (OD) of the stained cells is then correlated with cell migration.

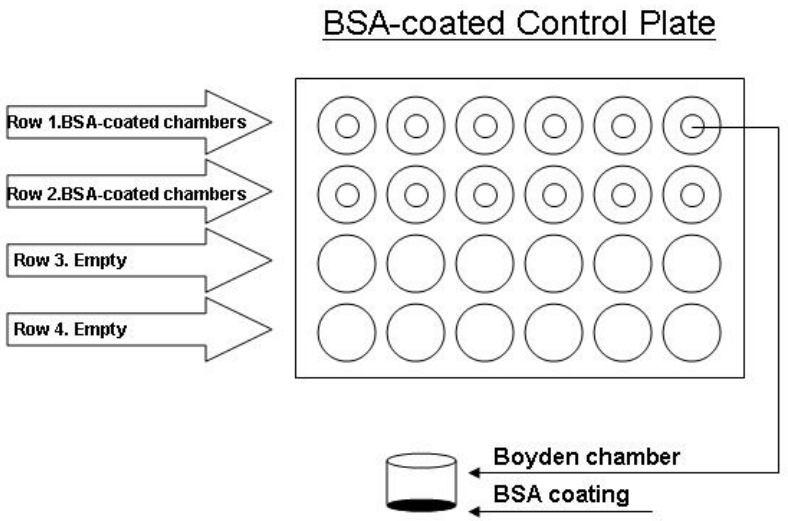
Overview of Millipore QCM™ 3µm Endothelial Cell Migration Assay – Fibronectin, Colorimetric.



Test Plate Layout:



Control Plate Layout:



Cell Harvesting

Prepare subject cells for investigation as desired. The following procedure is a suggested protocol 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). Wash the cell once with PBS and serum starve the cells in the basal media (without serum or growth supplements) containing 0.5% BSA for 12-18 hrs.
2. Wash cells once with sterile Harvest buffer (see Materials Not Supplied).
3. Add 3 mL Harvesting Buffer per100 mm dish and incubate at 37°C for 5-15 minutes.
4. Pipet cells off dish gently and add to 10 mL Quenching Medium (see Additional Materials Required) to inactivate trypsin/EDTA from Harvesting Buffer.
5. Centrifuge cells gently to pellet (1500 RPM, 5-10 minutes).
6. Gently resuspend pellet in 10 mL Quenching Medium, depending upon the size of the pellet.
7. Count cells and bring to a volume that gives 1.0×10^6 cells per mL.
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°C) prior to initiating assay.
2. Prepare a cell suspension containing 1.0×10^6 cells/mL according to cell harvesting instructions above.
3. In a laminar flow hood, remove the cover from the Test and Control plates and 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.*

4. Add 200 µL of the cell suspension from step #2 to **both** the Fibronectin-coated Boyden chambers of the Test Plate, and to the BSA-coated Boyden chambers of the Control Plate. The latter will serve as a migration control for the test samples.

5. Cover plates and incubate for 2 - 24 hours in a 37°C in a CO_2 incubator (4-6% CO_2).

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 by pressing the head of the swab firmly against a clean surface. These will be used to remove cells from Boyden chambers.

2. Using a clean disposable pipette tip for each well, remove medium from the Boyden chambers in the test and control plates. Use the included forceps to grasp the chamber and gently remove from the migration plate. Without touching the underside of the membrane, hold the chamber between thumb and forefinger and gently swab out media and cells from the interior of the chamber. *Use care to not puncture the membrane. Thorough cleaning of non-migratory cells will significantly reduce the amount of background staining.*

3. Transfer the chambers to a clean, empty well of the 24-well migration plate. Add 300 µL of Cell Stain Solution (~6 drops) to each well. Incubate for 15 minutes at room temperature.

4. Using the forceps, grasp and remove the chambers. Wash excess stain from the chamber by gently dipping several times into distilled water. Flick off excess water and allow to air dry.

5. Open and remove the included Stain Extraction Plate. Add 200 µL of Stain Extraction Solution to the required number of wells.

6. Transfer the chambers to the Stain Extraction Solution. Incubate for 5 – 10 minutes at room temperature on an orbital shaker.

7. Transfer 100 µL to the included 96-well microtiter plate Stain Quantification plate and read absorbance 540 – 570 nm.

***Note**: After extraction of the stain, if results suggest that individual cell counting is recommended, it is possible to re-stain and count individual cells by repeating the staining steps in the protocol.*

Optional step: Prior to Step 5, turn the chamber upside down and examine stained cells on the bottom of the membrane. If an inverted microscope is available, place upright chambers into empty third row of wells for examination. Count cells if desired and record data. **Note**: A graduated ocular (calibrated) or similar grid can be used to determine the number of cells per mm^2. Alternatively, automated equipment can count the entire bottom of the chamber. Cell counting may be used as an additional method of confirmation for comparison with final OD readings; however optical density (OD) is an accurate reflection of cell migration and individual cell counting is not required for many applications.

Calculation of Results

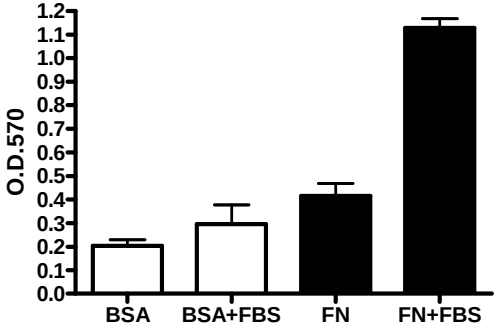
Results of the assay may be illustrated graphically by plotting the OD at ~570nm using a bar chart. A typical cell migration experiment will compare BSA-coated (negative) control chamber migration with Fibronectin-coated chamber migration. Additional migration may also be induced or inhibited in test wells through the addition of cytokines or other pharmacological agents.

BSA-coated control chambers function to determine the level of migration without stimulus. Cell migration in these chambers is generally low, and these chambers are typically used as "blanks" for interpretation of data. As such, migration in test wells can be described as the value of fibronectin-induced cell migration less the amount of migration visualized in the BSA-coated control.

When the stain extraction method of cell counting is used, a small amount of background staining, or "noise" is obtained from the staining of the membrane and the chamber itself. Thus, staining that is not significantly different from the BSA control chambers should be considered "background" staining.

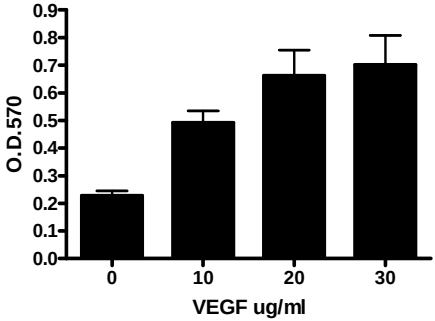
Sample Results

Figure1.



Migration assays were conducted using HUVEC cells ($2x10^5$ cells/well) in the Millipore QCM™ 3µm Endothelial Cell Migration Assay kit. FBS was used as a chemoattractant. Cells were allowed to migrate for 18 hrs and then stained according to the Assay Instructions. Cell migration was measured by plotting the OD at a wavelength of 570 nm. Data represents the mean ±S.D, n=3.

Figure 2.



Migration assays were conducted using HUVEC cells ($2x10^5$ cells/well) in the Millipore QCM™ 3µm Endothelial Cell Migration Assay kit. A different concentration of VEGF was used as a chemoattractant. Cells were allowed to migrate for 18 hrs and then stained according to the Assay Instructions. Cell migration was measured by plotting the OD at a wavelength of 570 nm. Data represents the mean ±S.D, n=3.

Warranty

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