# QCM™ Haptotaxis Cell Migration Assay - Fibronectin, Colorimetric

**Cat. No. ECM580**

**Sufficient for analysis of 12 samples**

**FOR RESEARCH USE ONLY**
**Not for use in diagnostic procedures**

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## Introduction

Cell migration is a fundamental function of normal cellular processes, including embryonic development, angiogenesis, wound healing, immune response, and inflammation. It has been reported that increased cell migration in a Boyden Chamber system correlates with increased invasive properties *in vivo*. [J Cell Biol 1998 140:961-972].

Cell migration is governed by a variety of factors, including cell surface adhesion receptor binding to extracellular matrix (ECM) proteins. One such matrix protein is Fibronectin (FN). FN is a widely distributed high molecular weight glycoprotein found in most extracellular matrices and blood plasma that is known to promote cell adhesion and affect cell morphology, migration, differentiation, and cytoskeletal organization.

MILLIPORE QCM™ Haptotaxis Cell Migration Assay – Fibronectin, Colorimetric provides a quick and efficient system for quantitative determination of various factors on cell migration, including screening of pharmacological agents, evaluation of integrins or other adhesion receptors responsible for 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 a hollow plastic chamber, sealed at one end with a porous membrane. This chamber is suspended over a larger well which may contain medium and/or chemoattractants. Cells are placed inside the Chamber and allowed to migrate through the pores, to the other side of the membrane. 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™ Haptotaxis Cell Migration Assay – Fibronectin, Colorimetric utilizes an 8 µm pore size, as this is appropriate for most cell types. This pore size supports optimal migration for most epithelial and fibroblast cells. The 8 µm pore size is not appropriate for lymphocyte 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™ Haptotaxis Cell Migration Assay – Fibronectin, Colorimetric assay measures haptotaxis - cell movement towards an immobilized extracellular matrix (ECM) protein gradient, where the bottom (outside) of the chamber has been coated with an ECM protein. The assay chambers have been pre-coated with human fibronectin or BSA (control) to allow optimal cell migration.

The QCM™ pre-coated Boyden chambers reduce assay time by up to 80% by eliminating the overnight coating step required in traditional assays. In addition, the assay 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™ Haptotaxis Cell Migration Assay – Fibronectin, Colorimetric is ideal for the study of epithelial and fibroblast cell haptotaxis. 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 8 μm pore size of this assay's Boyden chambers is not appropriate for the study of lymphocyte migration. The MILLIPORE QCM™ Haptotaxis 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. 2005689) 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. 2005791) 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.*

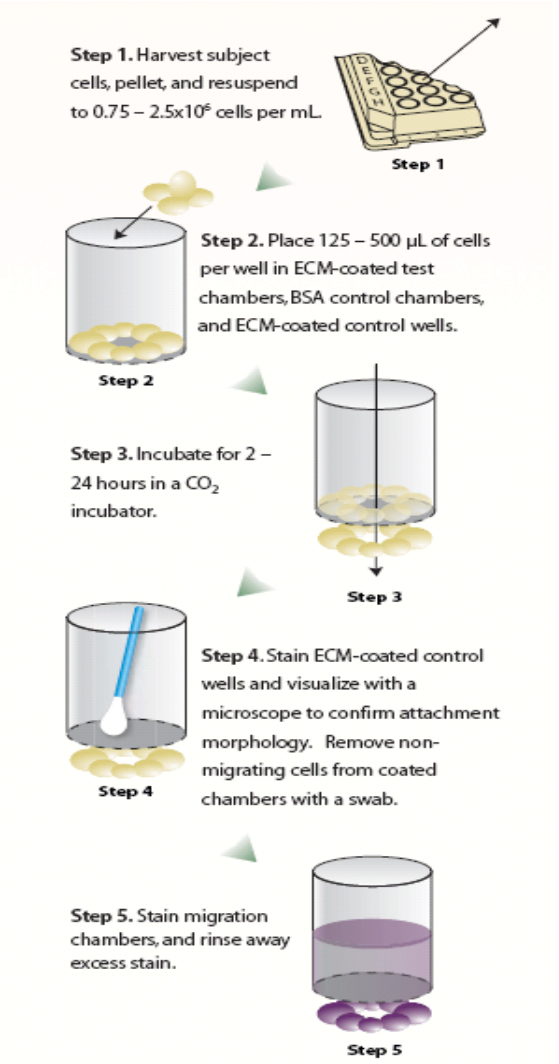
## Materials Not Supplied

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. Tissue culture growth medium appropriate for subject cells, such as DMEM containing 5% FBS.
4. 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.
5. Sterile PBS or HBSS to wash cells.
6. Distilled water
7. (Optional) Chemoattractant or pharmacological agent added to culture medium.
8. Low speed centrifuge and tubes for cell harvesting.
9. $CO_2$ incubator appropriate for subject cells.
10. Hemocytometer or other means of counting cells.
11. Trypan blue or equivalent viability stain.
12. Microplate reader (540-570 nm detection) or spectrophotometer.
13. Sterile cell culture hood
14. (Optional) Graduated ocular (calibrated), or automated method for counting stained cells on a membrane.
15. Shaker

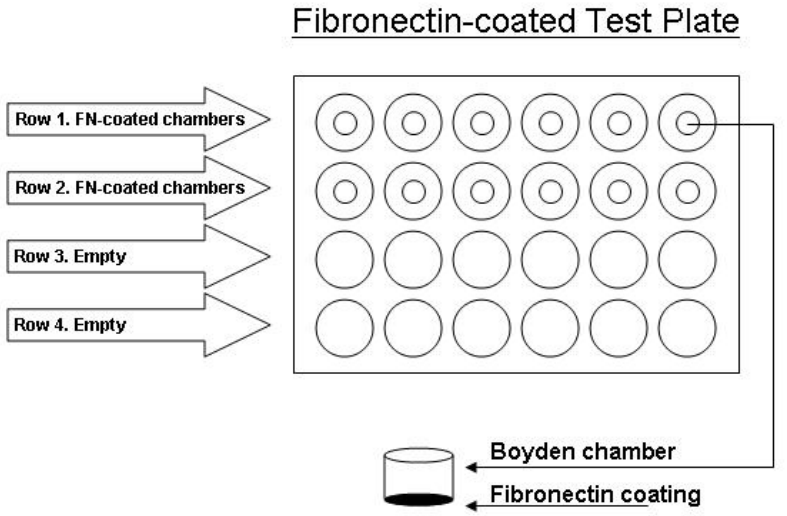
## Assay Principle

The MILLIPORE QCM™ Haptotaxis 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 8 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.

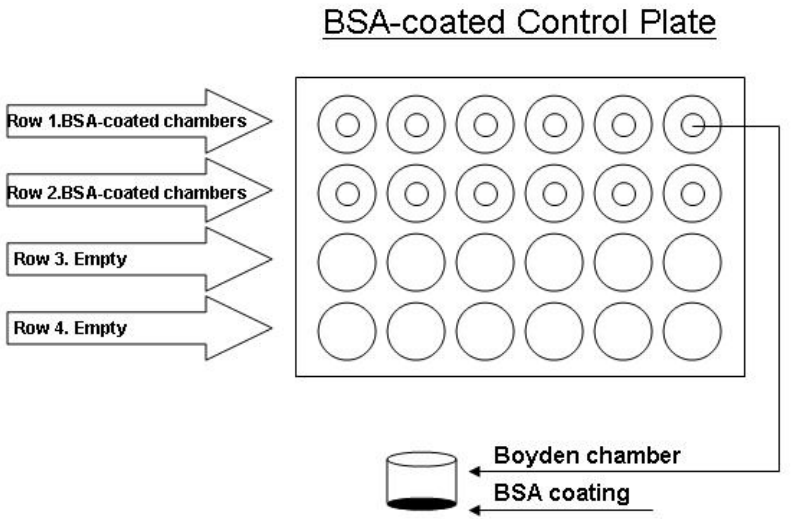
# Overview of QCM™ Haptotaxis Colorimetric Assay



**Test Plate Layout:**




**Control Plate Layout:**



## Cell Harvesting

Prepare subject cells for investigation as desired. The following procedure is a suggested protocol for HT1080 cells and may be optimized to suit individual cell types.

1. Use cells which have been passaged 2-3 times prior to the assay and are 80% confluent. Visually inspect cells before harvest, taking note of relative cell numbers and morphology. *(If desired, overnight serum starvation may be performed).*
2. Wash cells 2 x with sterile PBS or HBSS.
3. Add 5 mL Harvesting Buffer (see Materials Not Supplied) per 100 mm dish and incubate at 37°C for 5-15 minutes.
4. Pipet cells off dish gently and add to 10-20 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 1-2mL Quenching Medium, depending upon the size of the pellet.
7. Count cells and bring to a volume that gives 0.5 – 1.0 x $10^6$ 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 0.5 - 1.0 x $10^6$ 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 500 µL of serum-free media 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 300 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 350 µL of Cell Stain Solution (~6 drops) to each well. Incubate for 10 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.

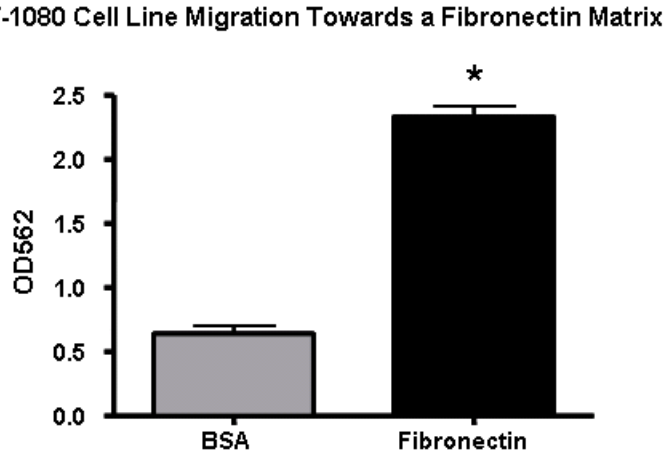
## Calculation of Results

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




Migration of HT-1080 cells towards a fibronectin matrix was assayed using the Haptotaxis Cell Migration Assay. Cells were incubated and then stained according to the Assay Instructions. Cell migration was measured by plotting the OD at a wavelength of 562nm. Values represent mean of three separate experiments ± standard error of the mean. **P*<0.001 versus migration in BSA-coated control chambers.

## Warranty

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