QCM™ 24-Well Colorimetric Cell Migration Assay

Cat. No. ECM 508

Sufficient for analysis of 24 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. Microporous membrane inserts are widely used for cell migration and invasion assays. The most widely accepted of which is the Boyden Chamber assay. Recently, a fluorescence blocking membrane insert was introduced to address these issues; however, this approach requires labeling of the cells with Calcein-AM and extensive washing to remove free Calcein before cell migration. The effect of this treatment on cell behavior/migration remains questionable.

The MILLIPORE QCM™ 24-well Cell Migration Assay (ECM508) eliminates cell pre-labeling and manual counting. The 24-well insert and colorimetric detection format allows for quantitative comparison of multiple samples.

In the MILLIPORE QCM™ 24-well Migration Assay, cells that have migrated to the bottom of the insert membrane are stained. The stain is then extracted and transferred to a 96-well microtiter plate for colorimetric measurement.

The MILLIPORE QCM™ 24-well Migration Assay 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, or analysis of gene function in transfected cells.

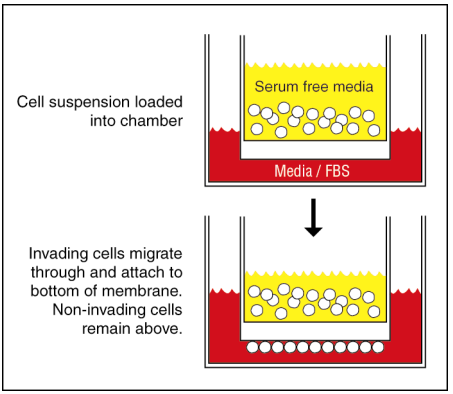
The MILLIPORE QCM™ 24-well Migration Assay utilizes an 8 µm pore size, as this is appropriate for most cell types. This pore size supports optimal migration for most epithelial and fibroblast cells; however, it is not appropriate for lymphocyte migration experiments. The system may be adapted to study different types of cell migration, including haptotaxis, random migration, chemokinesis, and chemotaxis.

In addition, Millipore continues to provide numerous migration, invasion, and adhesion products including:

- QCM™ 8µm 96-well Chemotaxis Cell Migration Assay (ECM510)
- QCM™ 5µm 96-well Chemotaxis Cell Migration Assay (ECM512)
- QCM™ 3µm 96-well Chemotaxis Cell Migration Assay (ECM515)
- QCM™ 96-well Cell Invasion Assay (ECM555)
- QCM™ 96-well Collagen-based Cell Invasion Assay (ECM556)
- 24-well Insert Cell Migration and Invasion Assay Systems
- CytoMatrix™ Cell Adhesion strips (ECM protein coated)
- QuantiMatrix™ ECM protein ELISA kits

Test Principle

The MILLIPORE QCM™ 24-well Cell Migration Assay is performed in a Migration Chamber, based on the Boyden chamber principle. Each kit contains 24 inserts; each insert utilizes an 8 µm pore size polycarbonate membrane, as this is appropriate for most cell types. This pore size supports optimal migration for most epithelial and fibroblast cells; however, it is not appropriate for lymphocyte migration experiments. Cells that have migrated through the polycarbonate membrane are incubated with Cell Stain Solution, then subsequently extracted and detected on a standard microplate reader (560 nm). The system may be adapted to study different types of cell migration, including haptotaxis, random migration, chemokinesis, and chemotaxis.



Application

The MILLIPORE QCM™ 24-well Migration Assay is ideal for the study of chemotaxis cell migration. Each Millipore Cell Migration Assay Kit contains sufficient reagents for the evaluation of 24 samples. The quantitative nature of this assay is especially useful for screening of pharmacological agents.

The MILLIPORE QCM™ 24-well Migration Assay is intended for research use only; not for diagnostic applications.

Kit Components

1. Sterile 24-well Cell Migration Plate Assembly: (Part No. 90333) Two 24-well plates with 12 inserts per plate (24 inserts total/kit).
2. Cell Stain: (Part No. 90144) One 20 mL bottle.
3. Extraction Buffer: (Part No. 90145) One 20 mL bottle.
4. Cotton Swabs: (Part No. 10202) 50 each.
5. Forceps: (Part No. 10203) One each.

Storage

Store kit materials at 2-8°C for up to their expiration date. Do not freeze.

Materials Not Supplied

1. Precision pipettes: sufficient for aliquoting cells.
2. Harvesting buffer: EDTA or trypsin cell detachment buffer. Suggested formulations include a) 2 mM EDTA/PBS, b) 0.05% trypsin in Hanks Balanced Salt Solution (HBSS) containing 25 mM HEPES, or other cell detachment formulations as optimized by individual investigators.

   Note: *Trypsin cell detachment buffer maybe required for difficult cell lines. Allow sufficient time for cell receptor recovery.*
3. Tissue culture growth medium appropriate for subject cells, such as DMEM containing 10% FBS.

4. Chemoattractants (eg. 10% FBS) or pharmacological agents for addition to culture medium, if screening is desired.
5. Quenching Medium: **serum-free** medium, such as DMEM, EMEM, or FBM (fibroblast basal media), containing 5% BSA.

   Note: *Quenching Medium* ***must contain*** *divalent cations (Mg^{2+}, Ca^{2+}) sufficient for quenching EDTA in the harvesting buffer.*
6. Sterile PBS or HBSS to wash cells.
7. Distilled water.
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 (560 nm).
13. 24-well tissue culture plate.
14. Sterile cell culture hood.

Cell Harvesting

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

1. Use cells that have been passaged 2-3 times prior to the assay and are 80% confluent.
2. Starve cells by incubating 18-24 hours prior to assay in appropriate serum-free medium (DMEM, EMEM, or equivalent).
3. Visually inspect cells before harvest, taking note of relative cell numbers and morphology.
4. Wash cells 2 times with sterile PBS or HBSS.
5. Add 5 mL Harvesting Buffer (see Materials Not Supplied) per 100 mm dish and incubate at 37°C for 5-15 minutes.

6. Gently pipette the cells off the dish and add to 10-20 mL Quenching Medium (see Materials Not Supplied) to inactivate trypsin/EDTA from Harvesting Buffer.
7. Centrifuge cells gently to pellet (1500 RPM, 5-10 minutes).
8. Gently resuspend the pellet in 1-5 mL Quenching Medium, depending upon the size of the pellet.
9. Count cells and bring to a volume that gives 0.5–1.0 x 10^6 cells per mL.
10. 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 and reagents to room temperature (23-25°C) prior to initiating assay.
2. Sterilize forceps with 70% ethanol and handle inserts with forceps.
3. Prepare a cell suspension containing 0.5-1.0 x 10^6 cells/mL in chemo-attractant-free media.
4. Add 300 µL of prepared cell suspension from step 3 to each insert.
5. Add 500 µL of serum free media in the presence or absence of chemo-attractant (e.g. 10% fetal bovine serum) to the lower chamber.

   Note: *Ensure the bottom of the insert membrane contacts the media. Air may get trapped at the interface.*
6. Cover plate and incubate for 4 - 24 hours at 37°C in a CO_2 incubator (4-6% CO_2).
7. Carefully remove the cells/media from the top side of the insert by pipetting out the remaining cell suspension, and place the migration insert into a clean well containing 400 µL of Cell Stain. Incubate for 20 minutes at room temperature.

8. Dip insert into a beaker of water several times to rinse.

9. While the insert is still moist, use a cotton-tipped swab to gently remove non-migratory cells layer from the interior of the insert. Take care not to puncture the polycarbonate membrane. Be sure to remove all cells on the inside perimeter, as any remaining cells inside the insert will contribute to background staining. Repeat procedure with a second, clean cotton-tipped swab.

10. Allow insert to air dry.

11. Transfer the stained insert to a clean well containing 200 µL of Extraction Buffer for 15 minutes at room temperature. Extract the stain from the underside by gently tilting the insert back and forth several times during incubation. Remove the insert from the well.

    Note: *Alternatively, cells can be counted manually through a microscope.*

12. Transfer 100 µL of the dye mixture to a 96-well microtiter plate suitable for colorimetric measurement.

13. Measure the Optical Density at 560 nm.

Calculation of Results

Results of the MILLIPORE QCM™ 24-well Cell Migration Assay may be illustrated graphically by the use of a "bar" chart. Samples without cells, but containing Cell Stain and Extraction Buffer are typically used as "blanks" for interpretation of data. A typical cell migration experiment will include control chamber migration without chemoattractant. Cell migration may be induced or inhibited in test wells through the addition of cytokines or other pharmacological agents.

The following figures demonstrate typical migration results. One should use the data below for reference only. This data should not be used to interpret actual assay results.

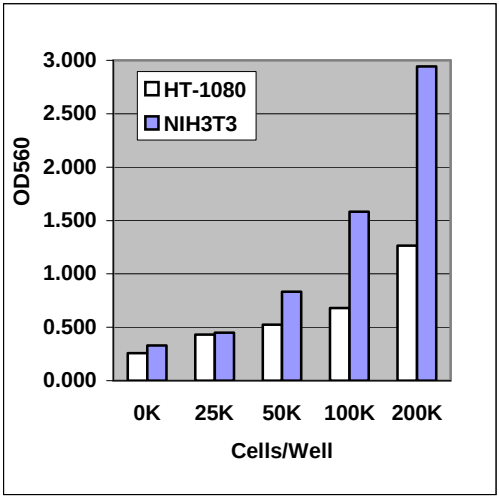


Figure 1: Cell Migration of HT-1080 vs. NIH3T3. HT-1080 and NIH3T3 cells were seeded at various concentrations and allowed to migrate toward 10% FBS for 4 hrs. Colorimetric measurements were taken according to *Assay Instructions*.

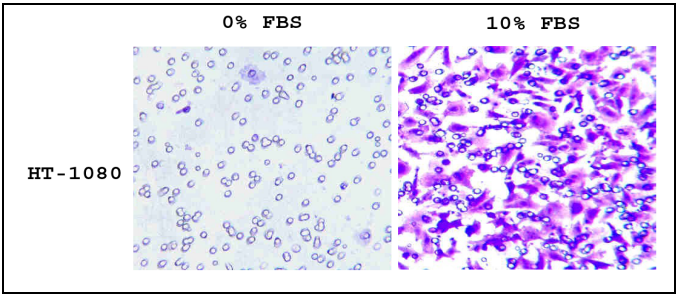


Figure 2: Cell Migration of HT-1080. HT-1080 cells were allowed to migrate towards 10% FBS for 4 hrs. 250,000 cells were used in each assay. Migrated cells on the bottom side of the membrane were stained according to *Assay Instructions*.

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