QCM™ Chemotaxis
96-Well Cell Migration Assay

Cat. No. ECM 510

Sufficient for analysis of 96 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. However, current methods of analysis are time-consuming and tedious, involving cotton swabbing of non-migrated cells on the top side of insert, manual staining and counting. 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 Chemicon QCM™ 96-well Migration Assay does not require cell labeling, scraping, washing or counting. The 96-well insert and homogenous fluorescence detection format allows for large-scale screening and quantitative comparison of multiple samples.

In the Chemicon QCM™ 96-well Migration Assay, migratory cells on the bottom of the insert membrane are dissociated from the membrane when incubated with Cell Detachment Buffer. These cells are subsequently lysed and detected by the patented CyQuant GR dye (Molecular Probes). This green-fluorescent dye exhibits strong fluorescence enhancement when bound to cellular nucleic acids.

The Chemicon QCM™ 96-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 Chemicon QCM™ 96-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, Chemicon also provides QCM™ 24-well insert cell migration assay systems, CytoMatrix™ Cell Adhesion strips coated with ECM proteins or anti integrin antibodies, and QuantiMatrix™ ECM protein ELISA kits.

Application

The Chemicon QCM™ 96-well Migration Assay is ideal for the study of chemotaxis cell migration. The quantitative nature of this assay is especially useful for large scale screening of pharmacological agents. The 8 μm pore size of this assay's Boyden chambers is appropriate for migration studies of most cell types. Each kit provides sufficient materials for the evaluation of 96 samples.

The Chemicon QCM™ 96-well Migration Assay is intended for research use only; not for diagnostic applications.

Kit Components

1. Sterile 96-well Cell Migration Plate Assembly: (Part No. 90128) One 96-well feeder tray, and one 96-well Cell Migration Chamber plate.
2. 96-well Cell Culture Tray: (Part No. 90129) One 96-well feeder tray.
3. Cell Detachment Solution: (Part No. 90131) One bottle – 16 mL.
4. 4X Cell Lysis Buffer: (Part No. 90130) One bottle – 16 mL.
5. CyQuant GR Dye®[1]: (Part No. 90132) One vial – 75 μL

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. Fluorescence plate reader.
13. Sterile cell culture hood.

Cell Harvesting

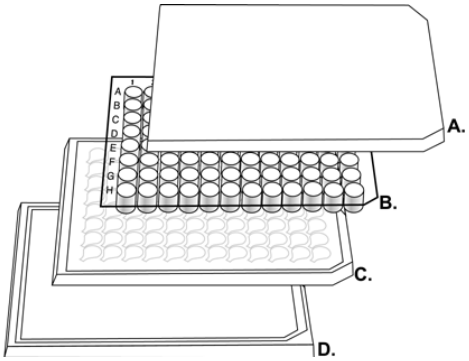
Prepare subject cells for investigation as desired. The following procedure is suggested and may be optimized to suit individual cell types.

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 pipet 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 0.25 – 1.0 x 10^6 cells per mL.

10. If desired, add additional compounds (cytokines, pharmacological agents, etc.) to cell suspension.

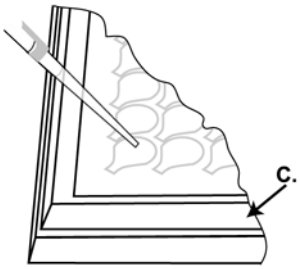
Assay Instructions

Perform the following steps in a tissue culture hood:



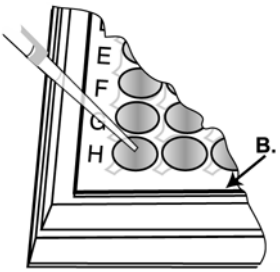
A. Lid **B.** Cell Migration Chamber Plate
C. 96-well Feeder Tray **D.** Base

1. For optimal results, bring plates and reagents to room temperature (25°C) prior to initiating assay. Remove lid of the migration chamber plate and add 150 µL of serum free media in the presence or absence of chemoattractant (e.g. 10% fetal bovine serum) to the wells of the feeder tray (lower chamber).



Step 1

2. After gently resuspending the cells, place 2.5 to 7.5 x 10^4 cells in 100 µL without chemoattractant into migration chamber.
3. Cover plate and incubate for 4 - 24 hours at 37°C in a CO_2 incubator (4-6% CO_2).



Step 2

4. Gently discard cells/media from the top side of the insert by flipping out the remaining cell suspension, and place the migration chamber plate onto the new 96-well feeder Tray containing 150 µL of prewarmed Cell Detachment Solution in the wells.

Incubate for 30 minutes at 37°C.

5. Dislodge cells completely from underside by gently tilting the migration chamber plate back and forth several times during incubation.
6. Prepare sufficient Lysis Buffer/Dye Solution for all samples. Dilute the CyQuant GR Dye 1:75 with 4X Lysis Buffer (eg. 4 µL dye in 300 µL of 4X Lysis Buffer) and add 50 µL of this Lysis Buffer/Dye Solution to each well of the feeder tray containing 150 µL cell detachment solution with the cells that migrated through the membrane. Incubate 15 minutes at room temperature.
7. Transfer 150 µL of the mixture to a new 96-well plate (not included) suitable for fluorescence measurement.
8. Read with a fluorescence plate reader using 480/520 nm filter set.

Calculation of Results

Results of the QCM™ Chemotaxis 96-well Cell Migration Assay may be illustrated graphically by the use of a "bar" chart. Samples without cells, but containing Cell Detachment Buffer, Lysis Buffer and CyQuant Dye 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.

Migratory cell number can be determined by running a fluorescent cell dose curve, as illustrated in Figure 4.

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.

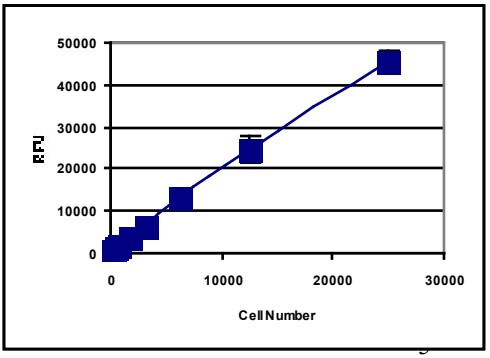


Figure 1: Quantitation of HT-1080 Using the CyQuant GR Dye. HT-1080 cells were resuspended in Cell Detachment Buffer; 150 µL of this cell suspension was mixed with 50 µL of 4X lysis buffer containing the fluorescence

dye. Fluorescence was determined as described in *Assay Instructions*.

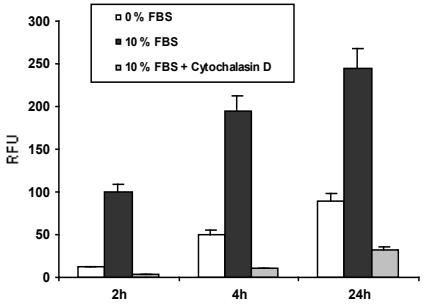


Figure 2: Human Fibrosarcoma HT-1080 Chemotaxis Assay. HT1080 chemotaxis toward 10% FBS was tested in the presence or absence of 10 µM Cytochalasin D. 40,000 cells were used in each assay. Fluorescence measurements were taken at 2, 4, or 24 hrs according to *Assay Instructions*.

References:

1. Jones LJ, Gray M, Yue ST, Haugland RP, Singer VL (2001), Sensitive determination of cell number using the CyQUANT cell proliferation assay, *J Immunol Methods* **254**, 85-98.
2. Gildea JJ, Harding MA, Gulding KM, Theodorescu D (2000), Transmembrane motility assay of transiently transfected cells by fluorescent cell counting and luciferase measurement, *Biotechniques* **29**, 81-86.

3. CyQUANT GR® is a registered trademark of Molecular Probes, Inc. The reagent is licensed from Molecular Probes, Inc. and is for use in kits sold by Chemicon International, Inc. for the monitoring of cell invasion and cell migration only.

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