MILLIPORE

QCM™ 96-Well
Cell Invasion Assay

Cat. No. ECM 555

Sufficient for analysis of 96 samples

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

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Introduction

Invasion through the extracellular matrix (ECM) is an important step in tumor metastasis. Cancer cells initiate invasion by adhering to and spreading along the blood vessel wall. Proteolytic enzymes, such as MMP collagenases, dissolve tiny holes in the sheath-like covering (basement membrane) surrounding the blood vessels to allow cancer cells to invade (1).

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-invaded cells on the topside 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 invasion. The effect of this treatment on cell behavior/invasion remains questionable.

The Millipore QCM™ 96-well Invasion 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 Millipore QCM™ 96-well Invasion Assay, invaded 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) (2-3). This green-fluorescent dye exhibits strong fluorescence enhancement when bound to cellular nucleic acids (4).

The MILLIPORE Cell Invasion Assay Kit provides an efficient system for evaluating the invasion of tumor cells through a basement membrane model. The kit utilizes ECMatrix™, a reconstituted basement membrane matrix of proteins derived from the Engelbreth Holm-Swarm (EHS) mouse tumor (5-8). We examined the kit's performance using human fibrosarcoma (HT-1080) and non-invasive fibroblasts (NIH3T3).

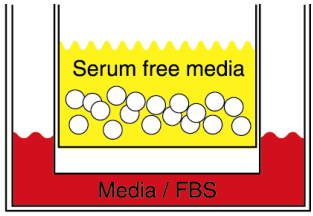
The Millipore QCM™ 96-well Invasion Assay provides a quick and efficient system for quantitative determination of various factors on cell invasion, including screening of pharmacological agents, evaluation of MMPs or other proteases responsible for cell invasion, or analysis of gene function in transfected cells.

In addition, Millipore also provides QCM™ 96-well Chemotaxis Cell Migration Assay Systems (8 µm or 3 µm pore), 24-well insert Cell Migration and Invasion Assay Systems, CytoMatrix™ Cell Adhesion strips coated with ECM proteins or anti-integrin antibodies, and QuantiMatrix™ ECM protein ELISA kits.

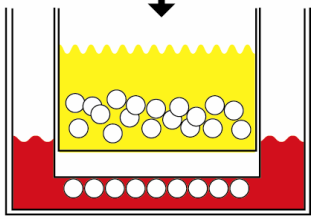
Test Principle

The MILLIPORE Cell Invasion Assay is performed in a 96 well invasion plate based on the Boyden chamber principle. This plate contains 96 inserts; each insert contains an 8 µm pore size polycarbonate membrane coated with a thin layer of ECMatrix™. The ECM layer occludes the membrane pores, blocking non-invasive cells from migrating through. Invasive cells, on the other hand, migrate through the ECM layer and cling to the bottom of the polycarbonate membrane. Invaded cells on the bottom of the insert membrane are dissociated from the membrane when incubated with Cell Detachment Buffer and subsequently lysed and detected by CyQuant GR dye.

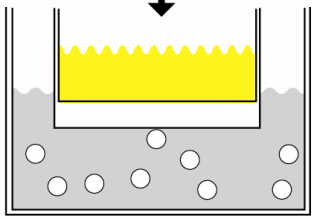
Cell suspension loaded into chamber



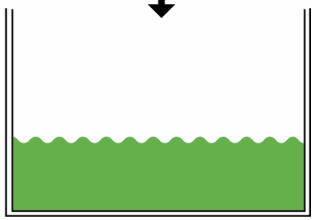
Invading cells migrate through and attach to bottom of membrane. Non-invading cells remain above.



Detach invaded cells in Cell Detachment Buffer



Lyse cells in Cell Lysis Buffer and detect cell numbers by CyQUANT® GR Dye*



Application

The MILLIPORE Cell Invasion Assay Kit is ideal for evaluation of invasive tumor cells. Each MILLIPORE Cell Invasion Assay Kit contains sufficient reagents for the evaluation of 96 samples. The quantitative nature of this assay is especially useful for large scale screening of pharmacological agents.

The MILLIPORE Cell Invasion Assay Kit is intended for research use only; not for diagnostic or therapeutic applications.

Kit Components

1. 96-well Cell Invasion Plate Assembly: (Part No. 90200) One 96-well feeder tray, and one 96-well Cell Invasion Chamber plate with ECMatrix™ –coated inserts.
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

Storage

Store kit materials at 2° to 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. Fluorescence plate reader.
13. Sterile cell culture hood.
14. Sterile PBS (Ca^{2+} and Mg^{2+} free)

Cell Harvesting

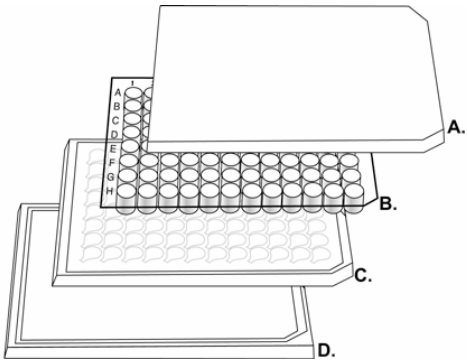
Prepare subject cells for investigation as desired. The following procedure is suggested for adherent cells only and may be optimized to suit individual cell types.

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 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.

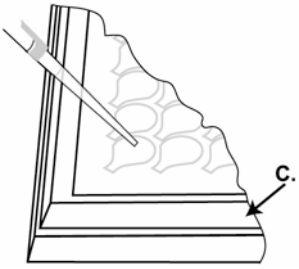
Assay Instructions

Perform the following steps in a tissue culture hood:

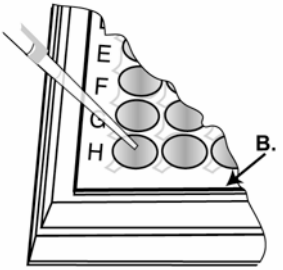


A. Lid **B.** Cell Invasion Chamber Plate
C. 96-well Feeder Tray **D.** Base

1. For optimal results, bring plates and reagents to room temperature (25°C) prior to initiating assay.
2. Add 100 µL of prewarmed serum free media to the interior of the inserts. Allow this to rehydrate the ECM layer for 1-2 hours at room temperature.
3. After rehydration from step 2, carefully remove media from the inserts without disturbing the ECMatrix™ – coated membrane.
4. 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 4



Step 5

5. After gently resuspending the cells, place 0.2 to 2.0 x 10^5 cells in 100 µL without chemoattractant into invasion chamber.

6. Cover plate and incubate for 12 - 24 hours at 37°C in a CO_2 incubator (4-6% CO_2).

7. Gently discard cells/media from the top side of the insert by flipping out the remaining cell suspension. Rinse the inserts by placing the chamber plate onto the new 96-well feeder tray containing 150 µL of PBS (Ca^{2+} and Mg^{2+} free). Incubate for 1 minute at room temperature.

8. Remove the invasion chamber plate from the feeder tray, and discard the PBS rinse. Place the invasion chamber plate back into the 96-well feeder tray (reusing the tray from the PBS rinse) containing 150 µL of prewarmed Cell Detachment Solution in the wells. Incubate for 30 minutes at 37°C.
9. Dislodge cells completely from underside by gently tilting the invasion chamber plate back and forth several times during incubation.
10. 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 invaded through the ECMatrix™-coated membrane. Incubate 15 minutes at room temperature.
11. Transfer 150 µL of the mixture to a new 96-well plate (not included) suitable for fluorescence measurement.
12. Read with a fluorescence plate reader using 480/520 nm filter set.

Calculation of Results

Results of the QCM™ 96-well Cell Invasion 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 invasion experiment will include control chamber migration without chemoattractant. Cell invasion may be induced or inhibited in test wells through the addition of cytokines or other pharmacological agents.

Invaded cell number can be determined by running a fluorescent cell dose curve, as illustrated in Figure 1.

The following figures demonstrate typical migration results. PE Cytofluor® 4000 with 480/520 nm filter set and gain setting of 65 was used. One should use the data below for reference only. This data should not be used to interpret actual assay results.

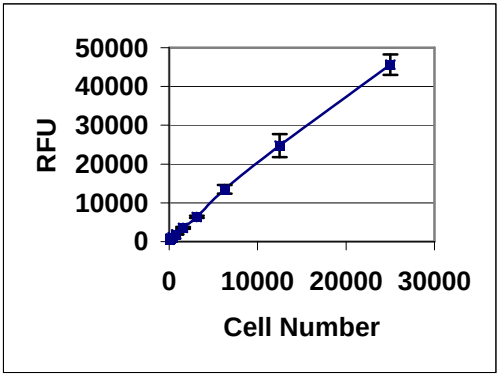


Figure 1: Quantitation of HT-1080 Using the CyQuant GR Dye. HT-1080 cells were resuspended in Cell Detachment Buffer; 150 µL of this cell suspension was mixed with 50 µL of 4X lysis buffer containing the fluorescence dye. Fluorescence was determined as described in *Assay Instructions.*

A **B**

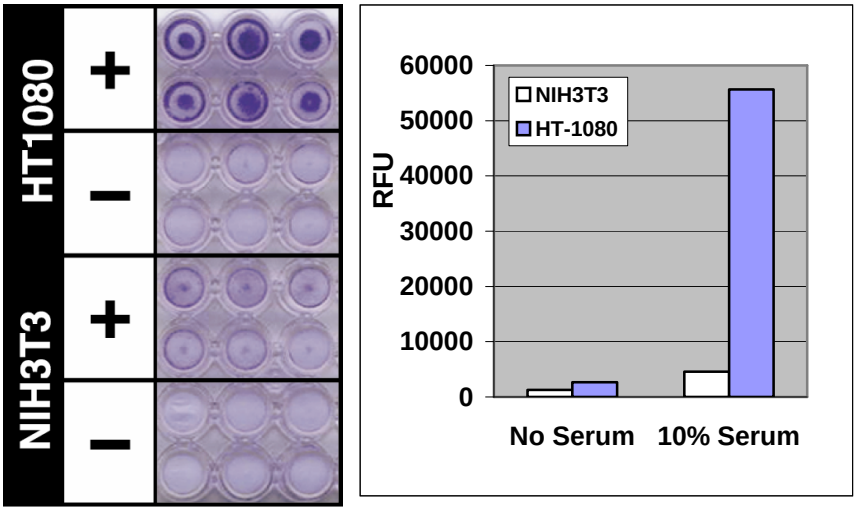


Figure 2: Human Fibrosarcoma HT-1080 Invasion Assay. HT1080 cells were allowed to invade toward 10% FBS for 12 hrs. 100,000 cells were used in each assay. **A:** Invaded cells on the low side of the membrane were stained with 0.1% Crystal violet solution. **B:** Fluorescence measurements were taken according to *Assay Instructions*.

References:

1. Egeblad M and Werb Z. (2002), New functions for the matrix metalloproteinases in cancer progression, *Nat Rev Cancer* **2**:161-74.
2. 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 Millipore International, Inc. for the monitoring of cell invasion and cell migration only.
3. Gildea JJ, Harding MA, Gulding KM, and Theodorescu D (2000), Transmembrane motility assay of transiently transfected cells by fluorescent cell counting and luciferase measurement, *Biotechniques* **29**, 81-86.
4. Jones LJ, Gray M, Yue ST, Haugland RP, and Singer VL (2001), Sensitive determination of cell number using the CyQUANT cell proliferation assay, *J Immunol Methods* **254**, 85-98.
5. Albini, A., Iwamoto, Y., Kleinman, H.K., Martin, G.R., Aaronson, G.R., Kozlowski, J. M., and McEwan, R.N. (1987). A rapid in vitro assay for quantitating the invasive potential of tumor cells, *Cancer Res.* **47**, 3239-3245.
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7. Terranova, V.P., Hujanen, E.S., Loeb, D.M., Martin, G.R., Thornburg, L., and Glushko, V. (1986) Use of reconstituted basement membrane to measure cell invasiveness and select for highly invasive tumor cells, *Proc. Natl. Acad. Sci. USA* **83**, 465-469.
8. Liotta, L.A. (1984) Tumor invasion and metastasis: role of the basement membrane, *Am. J. Pathol.* **117**, 339-348.

Warranty

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