User Manual

QCM™ 24-Well Collagen-Based Cell Invasion Assay (Colorimetric)

ECM551

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures. Not for human or animal consumption.

Product Overview

Introduction

Penetration of the subendothelial basement membrane marks a critical turning point in the metastatic process. As proliferating neoplastic cells attempt to escape the primary tumor site, local invasion of the surrounding tissue (interstitial stroma) must occur. Neovascularization is initiated by expression of angiogenic factors (for example, FGF, VEGF, HGF), providing nutritional requirements and access to the vascular system. Prior to penetrating the blood vessel endothelium and gaining access to the blood stream (intravasation), cancer cells must invade local tissues by degrading ECM components and ultimately, transverse the basement membrane. Once in circulation, these cells can form metastatic colonies at secondary locations, making this membrane a key invasive barrier.

The basement membrane surrounding the blood vessel endothelium is a thin, specialized network of extracellular matrix proteins (ECM) that serves many functions. Comprised of proteins and proteoglycans, such as collagen, laminin, entactin, fibronectin, heparin sulfate and perlecan, this membrane acts as a physical barrier between the epithelium and underlying tissues. It provides cell surface anchorage (via integrins, receptor kinases, and cell surface proteoglycans), induces cellular differentiation, gives architectural support, and limits the migration of normal cells. The ability of tumor cells to degrade the ECM components of the basement membrane and surrounding tissues is directly correlated with metastatic potential. By releasing proteolytic enzymes (for example, MMP collagenases, plasminogen activators, cathepsins), cancer cells are able to breach the membrane and penetrate the blood vessel wall. Collagen, the primary structural element of the basement membrane and tissue scaffolding protein, represents the main deterrent in the migration of tumor cells.

The ability to study cell invasion through a collagen barrier is of vital importance for developing possible metastatic inhibitors and therapeutics. The QCM™ 24-well Collagen-based Invasion Assay (ECM551) provides an efficient, *in vitro* system for quantitative analysis of tumor cell invasion.

The QCM™ 24-well Collagen-based Invasion Assay (ECM551) eliminates cell pre-labeling and manual counting. The 24-well insert and colorimetric detection format allows for quantitative comparison of multiple samples.

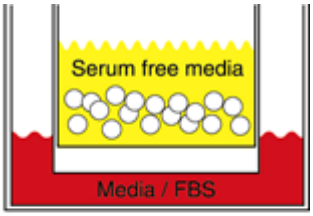
In addition, we provide diverse migration, invasion, and adhesion assay products including:

- QCM™ 8 µm 96-well Chemotaxis Cell Migration Assay (ECM510)
- QCM™ 5 µm 96-well Chemotaxis Cell Migration Assay (ECM512)
- QCM™ 3 µm 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

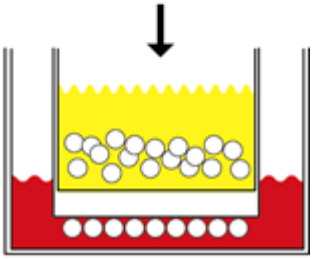
Test Principle

The Cell Invasion Assay is performed in an Invasion Chamber, based on the Boyden chamber principle. Each kit contains 24 inserts; each insert contains an 8 µm pore size polycarbonate membrane coated with a thin layer of polymerized collagen. The collagen layer occludes the membrane pores, blocking non-invasive cells from migrating through. Invasive cells, on the other hand, migrate through the polymerized collagen layer and cling to the bottom of the polycarbonate membrane. Invaded cells on the bottom of the insert membrane are incubated with Cell Stain Solution, then subsequently extracted and detected on a standard microplate reader (560 nm).

Cell suspension loaded into chamber



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



Application

The Cell Invasion Assay Kit is ideal for evaluation of invasive tumor cells. Each Cell Invasion 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 Cell Invasion Assay Kit is intended for research use only; not for diagnostic or therapeutic applications.

Materials Provided

- Sterile 24-well Cell Invasion Plate Assembly (90248): Two 24-well plates with 12 collagen–coated inserts per plate (24 inserts total/kit)
- Cell Stain (90144): One 20 mL bottle
- Extraction Buffer (90145): One 20 mL bottle
- Cotton Swabs (10202): 50 each
- Forceps (10203): One each

Materials Required (Not supplied)

Reagents

- Harvesting buffer: EDTA or trypsin cell detachment buffer. Suggested formulations include:
    - 2 mM EDTA/PBS
    - 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.

- Tissue culture growth medium appropriate for subject cells, such as DMEM containing 10% FBS
- Chemoattractants (for example, 10% FBS) or pharmacological agents for addition to culture medium, if screening is desired
- 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.

- Sterile PBS or HBSS to wash cells
- Distilled water
- Trypan blue, or equivalent viability stain

Equipment

- Precision pipettes: sufficient for aliquoting cells
- Low speed centrifuge and tubes for cell harvesting
- CO_2 incubator appropriate for subject cells
- Hemocytometer, or other means of counting cells
- Microplate reader (560 nm)
- 24-well tissue culture plate
- Sterile cell culture hood

Storage and Stability

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

Protocol

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 0.5-1.0x10^6 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 (25 °C) prior to initiating assay.
11. Sterilize forceps with 70% ethanol and handle inserts with forceps.
12. Add 300 µL of prewarmed serum-free media to the interior of the inserts. Allow this to rehydrate the ECM layer for 15-30 minutes at room temperature.
13. After rehydration from step 3, carefully remove 250 µL of media from the inserts without disturbing the membrane.
14. Prepare a cell suspension containing 0.5-1.0x10^6 cells/mL in chemo-attractant-free media.
15. Add 250 µL of prepared cell suspension from step 5 to each insert.
16. Add 500 µL of serum-free media in the presence or absence of chemo-attractant (for example, 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.
17. Cover plate and incubate for 24-72 hours at 37 °C in a CO_2 incubator (4-6% CO_2).
18. Carefully remove the cells/media from the top side of the insert by pipetting out the remaining cell suspension and place the invasion chamber insert into a clean well containing 400 µL of Cell Stain. Incubate for 20 minutes at 37 °C.
19. Dip insert into a beaker of water several times to rinse.
20. While the insert is still moist, use a cotton-tipped swab to gently remove non-invading cells/collagen 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.
21. Allow insert to air dry.
22. 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.
23. Transfer 100 µL of the dye mixture to a 96-well microtiter plate suitable for colorimetric measurement.
24. Measure the Optical Density at 560 nm.

Data Analysis

Calculation of Results

Results of the QCM™ 24-well Collagen-based Cell Invasion 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 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.

The following figure demonstrates typical invasion results. One should use the data below for reference only. This data should not be used to interpret actual assay results.

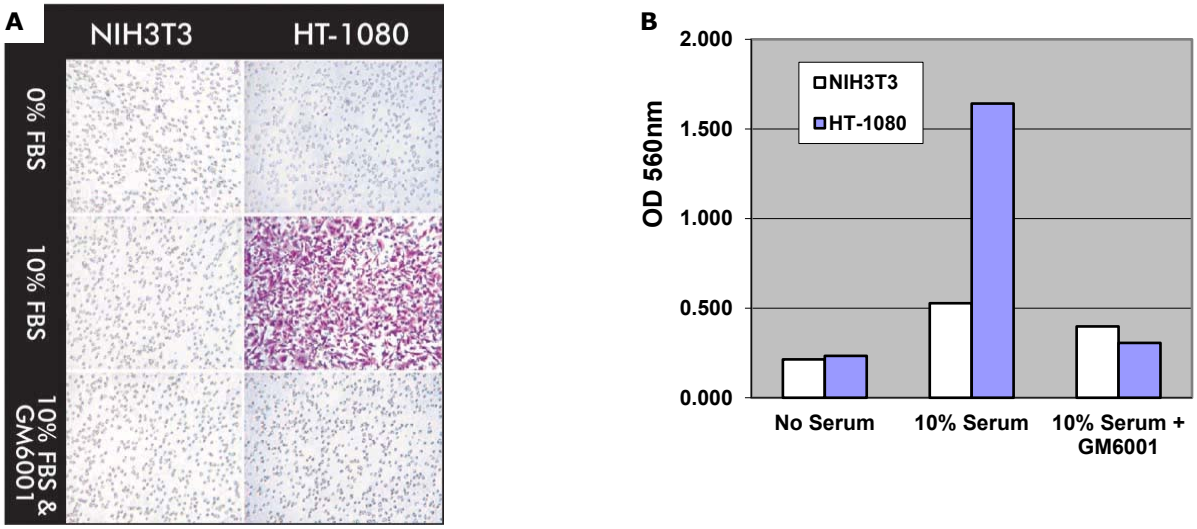


Figure 1: Cell Invasion of HT-1080 vs. NIH3T3. HT-1080 and NIH3T3 cells (+/-25 µM MMP Inhibitor GM6001) were allowed to invade toward 10% FBS for 24 hours. 250,000 cells were used in each assay. (**A**) Invaded cells on the bottom side of the membrane were stained according to Assay Instructions. (**B**) Colorimetric measurements were taken according to Assay Instructions.

References

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4. 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.
5. Liotta, L.A. (1984) Tumor invasion and metastasis: role of the basement membrane, Am. J. Pathol. 117, 339-348.

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