# QCM™ Laminin Migration Assay (24-well, colorimetric)

Catalog No. ECM220

**Sufficient for analysis of 24 samples**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures.

USA & Canada Phone: +1(800) 437-7500 Fax: +1 (951) 676-9209
www.millipore.com

## Introduction

Cell migration is a fundamental function of normal cellular processes, including embryonic development, angiogenesis, wound healing, immune response, and inflammation (1, 2).

Cell migration may be evaluated using several different methods; the most widely accepted being the Boyden Chamber assay. The Boyden Chamber system uses a two-chamber plate model in which a porous membrane provides an interface between two chambers. Cells are seeded in the upper chamber and chemoattractants placed in the lower chamber. Cells in the upper chamber migrate toward the chemoattractants by passing through the porous membrane to the lower chamber. Migratory cells are stained and quantified. Most cells are sized from 30 to 50 µm can migrate through 3 to 10 µm pore.

In addition to the Laminin Migration Assay, Millipore offers:

- Laminin, mouse purified (Cat. No. CC095)
- Accutase™ 100 mL bottle (Cat. No. SCR005)
- QCM 5µm Chemotaxis Assay 24-well-Colorimetric (Cat. No. ECM506)
- QCM 5µm Chemotaxis Assay 24-well-Fluorometric (Cat. No. ECM507)
- QCM 5µm Chemotaxis Assay 96-well-Fluorometric (Cat. No. ECM512)
- QCM ECMatrix™ Cell Invasion Assay, 96-well (8 µm), fluorometric (Cat. No. ECM555)
- QCM Collagen Cell Invasion Assay, 96-well (8 µm), fluorometric (Cat. No. ECM556)
- QCM Leukocyte Transendothelial Migration Assay – Colorimetric (Cat. No. ECM557)
- QCM Tumor Cell Transendothelial Migration Assay – Colorimetric (Cat. No. ECM558)
- Alpha/Beta Integrin-Mediated Cell Adhesion Colorimetric Array Combo Kit (Cat. No. ECM532)
- Alpha/Beta Integrin-Mediated Cell Adhesion Array Fluorometric Combo Kit (Cat. No. ECM535

*For Research Use Only; Not for use in diagnostic procedures*

## Application

The Millipore QCM Laminin Migration Assay incorporates a 5 µm porous membrane Boyden chamber and mouse laminin, a major extracellular matrix (ECM) protein in basement membrane, to examine cell migration dependent upon the presence of laminin. The laminin is isolated from mouse sarcoma and it is enriched with mouse laminin 1 (laminin-111). This ECM promotes cell adhesion, proliferation as well as migration in many cell types such as embryonic stem cells, neural cells, liver cells and cancer cells. Cells interact with laminin through integrins $\alpha 1\beta 1$, $\alpha 2\beta 2$, $\alpha 3\beta 1$, $\alpha 6\beta 1$, $\alpha 7\beta 1$, and $\alpha 6\beta 4$. The assay is designed specifically to examine the migration of a subset of fibroblasts, cancer cells and neural cells.

Laminin protein is coated on the bottom of each chamber's porous membrane as the driver for cell migration. A similarly BSA-coated control chamber(s) provides an appropriate migration control. Cells that migrate toward laminin are stained using crystal violet, a nucleic dye, extracted from the membrane surface, and quantified on a microplate reader using spectrophotometry.

Each kit provides sufficient materials for the evaluation of 24 samples.

## Kit Components

ECM220-1

1. 24-well Cell Migration Plate Assembly - 5 µm: (Part No. 2005708) Two 24-well plates, each containing 12- 5 µm pore inserts per plate.
2. BSA 30% solution: (Part No. CS203352) One vial containing 0.5 mL of a 30% solution.
3. Cell Stain Solution*: (Part No. 90144) One bottle.
4. Extraction Buffer: (Part No. 90145) One bottle.
5. Cotton Swabs: (Part No. 10202) Fifty each.
6. Forceps: (Part No. 10203) One each.
7. 24-well Stain Receiver Plate: (Part No. PIMWS2450) One each.
8. 96-well Stain Quantitation Plate: (Part No. 2005870) One each.

ECM220-2

1. Laminin: (Part No. CS208250) Two vials each containing 60 µL (1 mg/mL) in solution.

*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.

## Storage

ECM220 should be used within four months from date of receipt. In that time:

- ECM220-1 kit components should be stored at 2° to 8°C. DO NOT FREEZE.
- ECM220-2 component must be stored at -20°C until needed.

## Materials Not Supplied

1. Precision pipettes, sufficient for aliquoting appropriate volume of cells and reagents.
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) is recommended.

   ***Note**: Trypsin-based cell detachment buffer may be required for strongly adherent cell lines, but can strip cell surface proteins. Allow sufficient time for cell receptor recovery.*
3. Tissue culture growth medium appropriate for subject cells.
4. Quenching Buffer: Serum-free medium such as DMEM or RPMI-1640, containing 5% BSA

   ***Note**: Quenching Buffer must contain sufficient divalent cations ($Mg^{2+}$ or $Ca^{2+}$) to quench any EDTA present in the Harvesting Buffer.*
5. Chemoattractants (eg. 10% FBS) or pharmacological agents for addition to culture medium, if screening is desired.
6. Sterile PBS (Cat. No. BSS-1005-B) 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. Spectrophotometer (microplate reader with 540-570 nm detection capability).
13. Sterile cell culture hood.
14. (Optional) Graduated ocular (calibrated), or automated method for counting stained cells on a membrane.

## Coating Procedure

A coating concentration of 10 μg/mL mouse laminin performed optimally on internal analysis and is therefore the recommended concentration. Further optimization is at the discretion of the investigator.

Prepare laminin coating solution from stock and keep remaining stock solution at 2-8°C for up to one week or at -20°C for 6 months. *Perform the following steps in a tissue culture hood:*

1. To prepare a 10 μg/mL laminin coating solution for 12 inserts, thaw one vial of laminin at room temperature for 10 minutes.

2. Dilute one vial of laminin (60 μL) with 6 mL sterile PBS. Mix thoroughly by inverting the tube.

3. To prepare a 0.5% BSA solution for 12 inserts, dilute 0.1mL 30% BSA solution with 5.9 mL sterile PBS. Mix thoroughly by inverting the tube. For fewer BSA-coated control inserts, calculate volumes as needed.

4. Pipet 0.5 mL of either the laminin solution or BSA solution into the bottom of a **receiver** well within a plate assembly, NOT directly into the Boyden chamber porous membrane. Label the plate or lid to record coatings.

5. Place one Boyden chamber insert into each of the receiver wells containing a solution. Allow the inserts to coat overnight or up to 72 hours if necessary at 2-8°C or optionally, at 37°C for at least one hour.

6. Prior to performing the assay, using the forceps, rinse each coated insert with sterile PBS and dip each insert briefly in a 10 cm tissue culture plate filled with sterile PBS. Transfer each coated insert into a clean receiver well within the same 24-well plate assembly used for coating. These receiver wells should be filled with 0.5mL serum free medium containing 0.5% BSA and any desired conditional agent.

   ***Note**: Do not allow any insert to dry out as it may compromise the activity of the laminin.*

## Cell Harvesting

Prepare subject cells for investigation as desired. The following procedure is recommended for adherent cells and may be optimized to suit individual cell types. Perform the following steps in a tissue culture hood:

1. Use cells that are healthy and 80 to 90% confluent prior to the assay.
2. (Optional) Starve cells by incubating cells in starving medium (Serum-free medium with 0.3% to 1% BSA such as DMEM with 0.5% BSA) 18-24 hours prior to assay.
3. Visually inspect cells before harvesting, taking note of relative cell numbers and morphology.
4. Wash cells 2 times with sterile PBS or HBSS.
5. Add 5 mL of harvesting Buffer per 100 mm dish (see Materials Not Supplied) and incubate 5 to15 min at 37°C.
6. Gently pipette cells off the dish and add 10 mL Quenching Buffer to inactivate any trypsin/EDTA.
7. Gently centrifuge cells into pellet (1500 rpm, 5 to 10 min) and remove supernatant.
8. Resuspend cell pellet with 1 to 5 mL starving medium.
9. Count cells and bring to a volume of 0.5 - 1.0 x $10^6$ cells per mL.

## Assay Instructions

Prepare laminin- or BSA-coated inserts 24 to 72 hours according to the Coating Procedure. For each conditional agent or test compound, it is recommended to assay samples in triplicate on laminin-coated inserts and provide at least one BSA-coated insert.

*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.
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 chemoattractant-free media containing 0.5% BSA.
4. Add 200 µL of the cell suspension into each insert that is in contact with 500 µL or serum free media with 0.5% BSA in the presence or absence of test agent at the lower chamber.

   *Note: Air may get trapped at the interface. Ensure the bottom of the insert membrane contacts the media.*

5. Cover plate and incubate for 4 - 24 hours at 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. Carefully remove the cell/medium in the upper chamber by pipeting out the remaining suspension in the insert. Place the insert into a well within the 24-well Receiver Plate, containing 400 µL Cell Stain Solution. Incubate 10 to 20 min at room temperature.
2. Dip inserts into a beaker of water several times to rinse.
3. While the insert is still moist, use a cotton swap to remove any non-migratory cells from the interior of the insert. Carefully clean up all remaining cells to eliminate background stain. Use caution so as not to puncture the membrane while removing cells on the inside of perimeter. Repeat with a new cotton swap if needed.
4. Allow insert to air dry.
5. Transfer the insert to a new well containing 200 µL Extraction Buffer at room temperature for 15 min. Lightly shake the insert back and forth several times during incubation to extract stain from the membrane.

   ***Note**: Alternatively, stained cells can be counted under microscopy.*
6. Transfer 100 µL of the extracted stain from each well into the 96-well Quantitation Plate provided.
7. Measure the optical density at 570 nm. Alternatively, stained cells can be visualized using a light microscope.

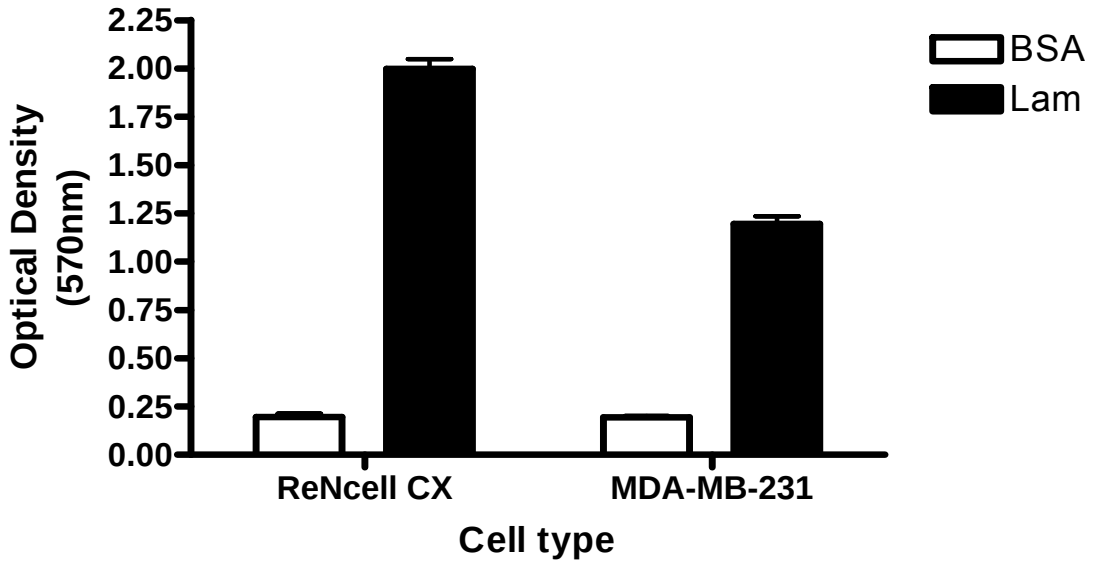
## Calculation of Results

Results of the Millipore Laminin Migration Assay may be illustrated graphically. Performing triplicates of each treatment is recommended to analyze the statistical significance of the outcome. A typical cell migration experiment will include appropriate controls including at least one control chamber coated with BSA and laminin-coated inserts without conditional reagents. Cell migration may be induced or inhibited in test wells through the addition of cytokines or other pharmacological agents.

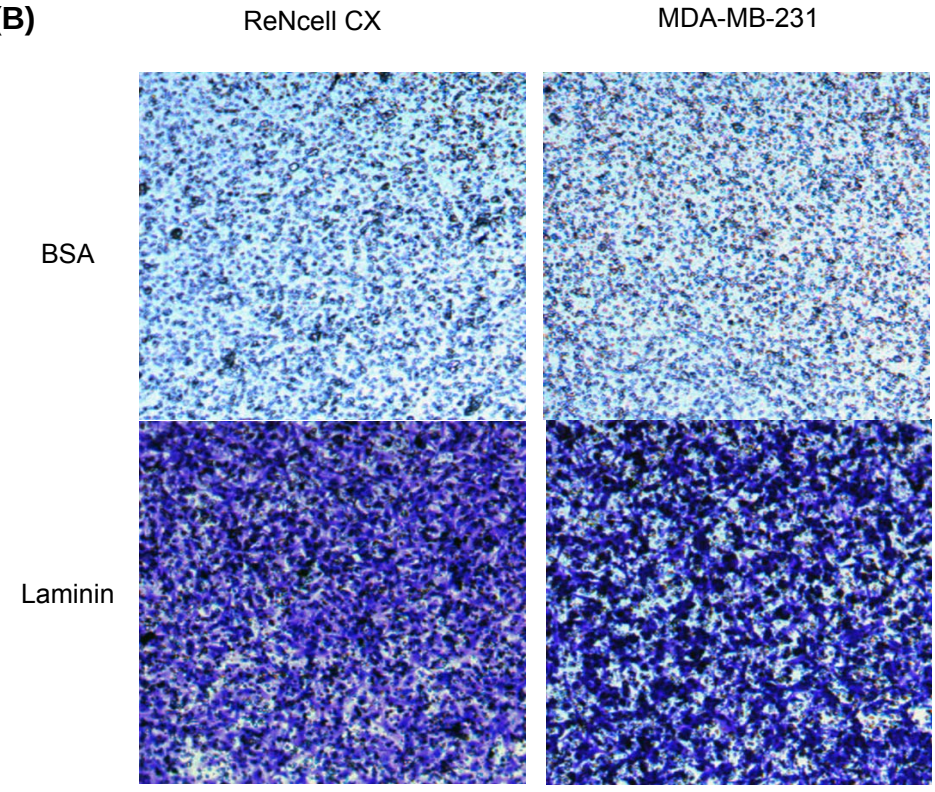
The data is for reference only and should not be used to interpret actual assay results. Data can vary based on the cell line assayed.

## Assay Example

**(A)**




**(B)**




Human cortical neural stem cell line ReNcell CX (Cat. No. SCC007) and breast cancer cell line MDA-MB-231 were resuspended in DMEM/F12 or DMEM with 0.5% BSA. MDA-MB-231 cells were starved overnight before harvesting. 250K cells were seeded per well and allowed to migrate for 4 hours at 37°C before staining and extraction. Figure **(A)** is a graphic depiction of a representative assay using triplicates on each cell type to show standard error. Open bar represents BSA-coated control well and closed bar describes laminin-coated or stimulated migration. Figure **(B)** provides a representative light microscopy view of the underside of the porous membrane.

## References


1. Clark, R.A.F., Tonnesen, M.G., Gailit, J., Cheresh, D.A. (1996). *Am. J. Pathol*. **148**:1407-1421.
2. Martin, P. (1997) *Science.* **276**, 75-81.


## Warranty

**Millipore Corporation** ("Millipore") warrants its products will meet their applicable published specifications when used in accordance with their applicable instructions for a period of one year from shipment of the products. **MILLIPORE MAKES NO OTHER WARRANTY, EXPRESSED OR IMPLIED. THERE IS NO WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE.** The warranty provided herein and the data, specifications and descriptions of Millipore products appearing in Millipore's published catalogues and product literature may not be altered except by express written agreement signed by an officer of Millipore. Representations, oral or written, which are inconsistent with this warranty or such publications are not authorized and if given, should not be relied upon.

In the event of a breach of the foregoing warranty, Millipore's sole obligation shall be to repair or replace, at its option, the applicable product or part thereof, provided the customer notifies Millipore promptly of any such breach. If after exercising reasonable efforts, Millipore is unable to repair or replace the product or part, then Millipore shall refund to the Company all monies paid for such applicable Product. **MILLIPORE SHALL NOT BE LIABLE FOR CONSEQUENTIAL, INCIDENTAL, SPECIAL OR ANY OTHER DAMAGES RESULTING FROM ECONOMIC LOSS OR PROPERTY DAMAGE SUSTAINED BY ANY COMPANY CUSTOMER FROM THE USE OF ITS PRODUCTS.**

Unless otherwise stated in our catalog or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans or animals.