Version 4a, Last updated 9 June 2025

ab235697
Cell Invasion Assay (Basement Membrane), 96-well, 8 µm

For the measurement of cell migration in response to stimuli.

This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview

Cell Invasion Assay (Basement Membrane), 96-well, 8 µm (ab235697) utilizes a Boyden chamber coated with Basement Membrane Extract (BME), where the cells invade the matrix and then migrate through a semi-permeable membrane in the Boyden chamber in response to stimulants or inhibitory compounds. The percent cell invasion can be analyzed directly in a plate reader. Our assay is easy to use, sensitive and adaptable to high-throughput systems.

Prepare cells.

Prior to the assay, starve cells for 18-24 hr in serum-free media.

Set up cell invasion assay containing desired chemoattractant in the bottom chamber. Incubate the Cell Chamber (96 x 8µm)/Cell Invasion Chamber at 37°C in CO_2 incubator for 2-48 hrs.

Prepare Standard Curve for each cell type.

Wash Cells.

Add Cell dye and incubate at 37°C in CO_2 incubator for 60 minutes.

Disassemble the Cell Chamber (96 x 8µm)/Cell Invasion Chamber, remove the top chamber and read the bottom well at Em/Ex = 530/590 nm.

2. Materials Supplied and Storage

Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Components are stable for 6 months after the first thaw.

Aliquot components in working volumes before storing at the recommended temperature.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Wash Buffer II (Sterile)	50 mL	-20°C	-20°C
Cell Dissociation Solution I	15 mL	-20°C	-20°C
Control Migration Inducer	300 µL	-20°C	-20°C
Cell Dye I	1.5 mL	-20°C	-20°C
Cell Migration Chamber, 96-Well (8 µm)	1	-20°C	-20°C
Basement Membrane Solution	4 x 1 mL	-20°C	-20°C

PLEASE NOTE: Cell Dissociation Solution I was previously labelled as Cell Dissociation Solution, and Cell Dye I as Cell Dye, and Cell Migration Chamber, 96-Well (8 µm) as Cell Chamber (96 x 8µm) and Cell Invasion Chamber, and Control Migration Inducer as Control Invasion Inducer, and Wash Buffer II (Sterile) as Wash Buffer II and Wash Buffer. The composition has not changed.

3. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Fluorescence Plate Reader.
- Cotton Swab.
- Centrifuge to spin 96-well plate.
- 96-well clear bottom white plate.

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements.

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

5.1 Wash Buffer II

1. Ready to use as supplied.
2. Bring to 37°C before use.
3. Stable for six months after the first thaw.

5.2 Cell Dissociation Solution I

1. Ready to use as supplied.
2. Bring to 37°C before use.
3. Stable for six months after the first thaw.

5.3 Control Migration Inducer

1. Ready to use as supplied.
2. Bring to 37°C before use.
3. Stable for six months after the first thaw.

5.4 Cell Dye I

1. Ready to use as supplied.
2. Aliquot and store at -20°C.
3. Bring to 37°C before use.

5.5 Cell Chamber (96 x 8µm)/Cell Invasion Chamber

1. Open under sterile conditions.

5.6 Basement Membrane Solution

1. Thaw vials as needed slowly on ice or in frost-free 4°C refrigerator. Temperatures above 4°C will rapidly turn the Base Membrane Solution into a gel. Thawing may take overnight at 4°C. The thawed matrix can be stored at 2-8°C for one week.
2. For long term (6 months) storage, we recommend aliquoting into several tubes according to use and storing at -20°C.

6. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.
- Assay all standards, controls and samples in duplicate.

6.1 Cell Invasion Assay Protocol:

1. Add 40 µL of Basement Membrane Solution to coat desired wells of the Top Chamber. Incubate plate at 37°C in an incubator for the Basement Membrane Solution to gel for 1 hour.
2. Grow cells of interest in desired media and culture conditions. Grow enough cells to perform a Cell Invasion Assay and a Standard Curve.
3. Adherent cells should be cultured to ~80% confluence.
4. Prior to the assay, starve cells for 18-24 hours in a serum-free media (0.5% serum can be used, if needed).
5. After starvation, harvest the cells and centrifuge at 1,000 x *g*, for 5 minutes to pellet cells.
6. Resuspend cell pellet in Wash Buffer II and count the number of cells using hemocytometer or automated cell counter.
7. Resuspend cells at 1 x 10^6 cells/mL in a serum-free media.
8. Under sterile conditions, disassemble the Cell Chamber (96 x 8µm)/Cell Invasion Chamber and carefully remove the plate cover and the top chamber.

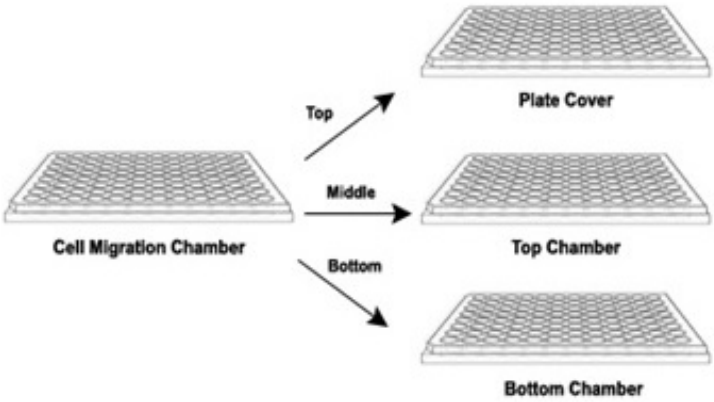

Figure 1. Cell Invasion plate: The cells are added to the Top Chamber and the Control Migration Inducer or chemoattractant are added to the Bottom Chamber.

9. **Bottom Chamber:** Add 200 µL of medium per well containing desired chemoattractant to the bottom chamber.
10. In control well(s), we recommend omitting the chemoattractant.
11. For Positive Control, add 20 µL of Control Migration Inducer to 180 µL of medium in the bottom chamber.
12. Reassemble the top and bottom chambers while ensuring no air bubbles are trapped between them
13. **Top Chamber:** Add 50 µL (50,000 cells) of cell suspension to each well of the top chamber.
14. Add desired stimulator or inhibitor to the top well, and gently mix.
15. Make up the volume to 100 µL with media. Carefully place the plate cover and incubate the Cell Migration Chamber at 37°C in CO_2 incubator for 2-48 hours.

6.2 Standard Curve:

1. Each cell type requires a separate Standard Curve. Prepare a Standard Curve by adding 50 µL cell suspension (1 x 10^6 cells/mL, 50,000 cells) per well in a 96-well plate (white plate clear bottom).
2. Serially dilute the cells 1:1 in Wash Buffer II and generate a Standard Curve of cells (50,000, 25,000, 12,500, 6,250, 3,125, 1,562 and 781) in 100 µL total volume.
3. As blank, use 100 µL of Wash Buffer II.
4. Add 10 µL of Cell Dye I to each well.
5. Incubate at 37°C for 1 hour.
6. Read the fluorescence at Ex/Em = 530/590 nm.
7. Plot the Standard Curve of Number of Cells Vs RFU obtained.
8. Fit the data points using a linear trendline with zero intercept.
9. The equation for the straight line and R-square value are used for data analysis of samples.

   Δ Note: The Cell Invasion RFU reading should fall in the linear range of the Standard Curve. We recommend using triplicates for Standard Curve.

6.3 Data Collection and Analysis:

1. After the desired incubation with cell invasion inducers/inhibitors, carefully remove the plate cover and aspirate media from the top chamber without puncturing the membrane and matrix.
2. Remove cells from the top chamber using a cotton swab. Disassemble the Cell Chamber (96 x 8µm)/Cell Invasion Chamber by removing the top chamber.
3. Invert the top chamber and set it aside. Place the plate cover on top of bottom chamber and centrifuge the plate at 1,000 x *g* for 5 minutes at room temperature.
4. Carefully aspirate the media from the bottom chamber, and wash the chamber with 200 µL Wash Buffer II.
5. Centrifuge the plate at 1,000 x *g* for 5 minutes at room temperature and aspirate the media from the bottom chamber.
6. For every eleven wells to be assayed, prepare a mix of 100 µL of Cell Invasion Dye in 1 mL of Cell Dissociation Solution I. Mix well.
7. Add 100 µL of the mix to each well of the bottom chamber.

8. Reassemble the Cell Chamber (96 x 8µm)/Cell Invasion Chamber by placing the top chamber into the bottom chamber. Incubate at 37°C in CO_2 incubator for 60 minutes.
9. Disassemble the Cell Chamber (96 x 8µm)/Cell Invasion Chamber, remove the top chamber and read the bottom well at Em/Ex = 530/590 nm.

   Δ Note: Invasive cells pass through the basement membrane and cling to the outer side of the top chamber. Non-invasive cells stay in the upper chamber.

   Δ Note: During incubation with Cell Dissociation Solution I/Cell Invasion Dye, gently tap the plate on the side to ensure optimal dissociation of the invasive cells that cling to the outer side of the top chamber.

7. Data Analysis

1. Calculate the number of cells invaded using the equation of the straight line obtained from Standard Curve.
2. Percentage Invasion can be calculated as follows:

$$\% \text{ Invasion} = \frac{\text{B\# Cells in Lower Chamber}}{\text{Total \# Cells added to Top Chamber}} * 100$$

8. FAQs / Troubleshooting

General troubleshooting points are found at www.abcam.com/assaykitguidelines.

9. Typical Data

Data provided **for demonstration purposes only.**

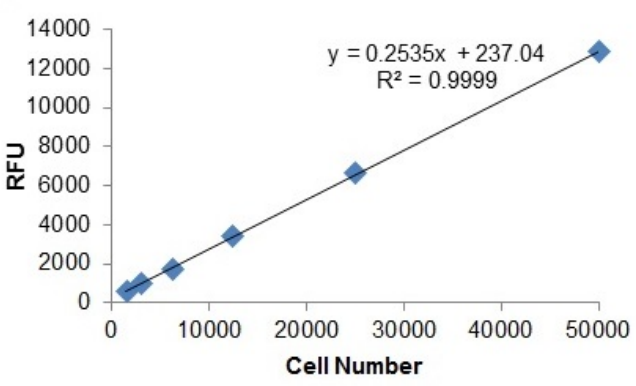

Figure 2. Standard Curve: HT-1080 cells were harvested, counted and serially diluted to obtain desired cell number. Cells were incubated according to the protocol with Cell Invasion Dye and fluorescence (Em/Ex 530/590) was measured.

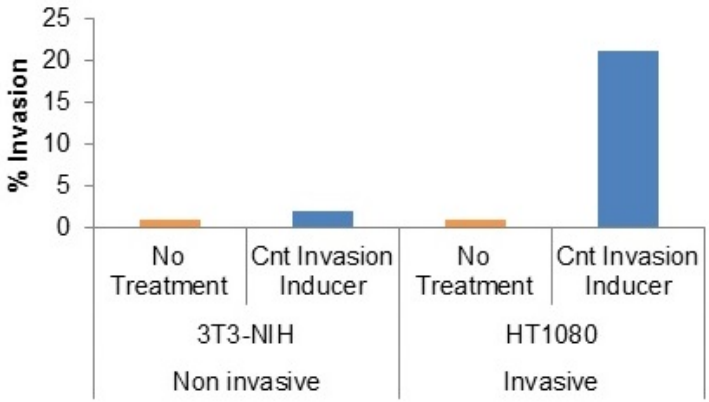

Figure 3. Cell Invasion: 3T3-NIH and HT-1080 cells were starved overnight and treated with Control Migration Inducer or remain untreated (No Treatment). Treatment with Control Migration Inducer demonstrated a significant increase in invasion of HT 1080 cells as compared to 3T3-NIH control cells.

10. Notes

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)