Cell Invasion Assay (Basement Membrane), 96-well, 8 µm (#BN01086)

(Catalog # BN01086; 100 assays; Store at -20°C)

I. Introduction:

Cell invasion is the ability of cells to migrate from one area to another through an extracellular matrix. Cell invasion is exhibited by both normal cells as well as cancerous cells in response to specific external signals, including chemical & mechanical stimuli. During invasion, extracellular matrix is enzymatically degraded by cellular proteases before cells migrate to the new location. Cell invasion is required for normal processes such as wound repair, vasculature formation and the inflammatory response as well as the abnormal invasion of tissues by tumor cells during metastasis. Assay Genie's Cell Invasion Assay Kit utilizes a Boyden chamber coated with Basement Membrane Extract (BME), where the cells invade the matrix and then migrate through a semipermeable 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.

II. Application:

- Measure cell invasion in response to stimuli
- Screen compounds that influence cell invasion

III. Sample Type:

- Invasive cell lines

IV. Kit Contents:

Components	BN01086	Cap Code	Part number
Wash Buffer	2 x 100 ml	NM	BN01086-1
Cell Dissociation Solution	10 ml	NM	BN01086-2
Control Invasion Inducer	1.5 ml	Red	BN01086-3
Cell Dye	1 ml	Blue	BN01086-4
Cell Invasion Chamber	1 each	Plate	BN01086-5
Basement Membrane Solution	4 x 1 ml	Green	BN01086-6

V. User Supplied Reagents & Equipment:

- Fluorescence Plate Reader
- Cell Culture Media
- Cotton Swabs
- Centrifuge to spin 96-well plate
- 96-well clear bottom white plate

VI. Storage and Reagents Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Assay is performed under sterile conditions. Read entire protocol before performing the experiment.

- **Cell Invasion Chamber:** Open under sterile conditions. Keep at Room temperature.
- **Basement Membrane Solution:** 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. **For long term (6 months) storage**, we recommend aliquoting into several tubes according to use and storing at -20°C.
- **Control Invasion Inducer, Cell Dissociation Solution and Wash Buffer:** Store at -20°C. Bring to 37°C before use. Stable for six months.
- **Cell Invasion Dye:** Aliquot and store at -20°C. Bring to 37°C before use.

VII. 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 hr.
2. Grow cells of interest in desired media and culture conditions. Grow enough cells to perform a Cell Invasion Assay and a Standard Curve. Adherent cells should be cultured to ~80% confluence. Prior to the assay, starve cells for 18-24 hrs in a serum-free media (0.5% serum can be used, if needed). After starvation, harvest the cells and centrifuge at 1,000 x g, for 5 min. to pellet cells. Resuspend cell pellet in Wash Buffer and count the number of cells using hemocytometer or automated cell counter. Resuspend cells at 1 x 10^6 cells/ml in a serum-free media.

   Under sterile conditions, disassemble the Cell Invasion Chamber and carefully remove the plate cover and the top chamber.

   Bottom Chamber: Add 200 µl of medium per well containing desired chemoattractant to the bottom chamber. In control well(s), we recommend omitting the chemoattractant. For Positive Control, add 20 µl of Control Invasion Inducer to 180 µl of medium in the bottom chamber. Reassemble the top and bottom chambers while ensuring no air bubbles are trapped between them.

   Top Chamber: Add 50 µl (~50,000 cells) of cell suspension to each well of the top chamber. Add desired stimulator or inhibitor to the top well, and gently mix. Make up the volume to 100 µl with media. Carefully replace the plate cover and incubate the Cell Invasion Chamber at 37°C in CO_2 incubator for 2-48 hrs.

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3. **Standard Curve**: Each cell type requires a separate Standard Curve. Prepare a Standard Curve by adding 50 µl cells (1 x 10^6 cells/ml, ~ 50,000 cells) per well in a 96-well plate (white plate clear bottom). Serially dilute the cells 1:1 in Wash Buffer and generate a Standard Curve with different cell # (50,000, 25,000, 12,500, 6,250, 3,125, 1,562 and 781) in 100 µl total volume. As blank, use 100 µl of Wash Buffer. Add 10 µl of Cell Invasion Dye to each well. Incubate at 37°C for 1 hr. Read the fluorescence at Em/Ex 530/590 nm. Plot the Standard Curve (Number of Cells Vs RFU) obtained. Fit the data points using a linear trend line with zero intercept. The equation for the straight line and R-squared value are used for data analysis of samples.

   Note:

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

4. **Data Collection and Analysis:** 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. Remove cells from the top chamber using a cotton swab. Disassemble the Cell Invasion Chamber by removing the top chamber. 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 min. at room temperature. Carefully aspirate the media from the bottom chamber, and wash the chamber with 200 µl Wash Buffer. Centrifuge the plate at 1,000 x g for 5 min. at room temperature and aspirate the media from the bottom chamber. For every eleven wells to be assayed, prepare a mix of 100 µl of Cell Invasion Dye in 1 ml of Cell Dissociation Solution. Mix well. Add 100 µl of the mix to each well of the bottom chamber. Reassemble the Cell Invasion Chamber by placing the top chamber into the bottom chamber. Incubate at 37°C in CO_2 incubator for 60 min. Disassemble the Cell Invasion Chamber, remove the top chamber and read the bottom well at Em/Ex = 530/590 nm. Calculate the number of cells invaded using the equation of the straight line obtained from Standard Curve. Percentage Invasion can be calculated as follows:

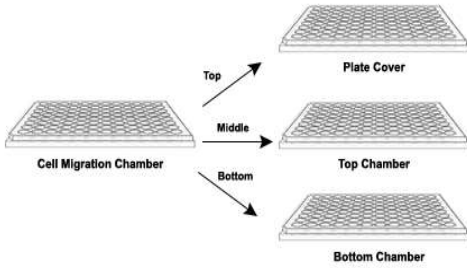
$$\% \textbf{ Invasion} = \left(\frac{\textbf{\# Cells in Lower Chamber}}{\textbf{Total \# Cells added to Top Chamber}}\right) \boldsymbol{x}\ \mathbf{100}$$

   Notes:

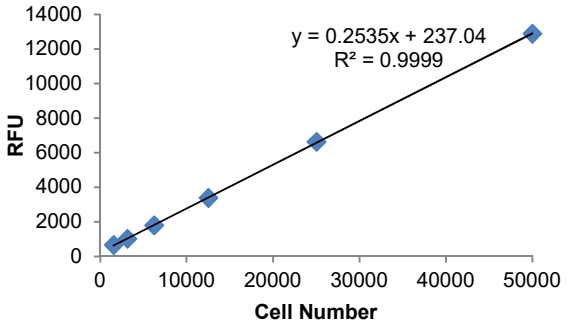
   a. Invasive cells pass through the basement membrane and cling to the outer side of the top chamber. Non-invasive cells stay in the upper chamber.

   b. During incubation with Cell Dissociation Solution/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.

A.



B.



C.

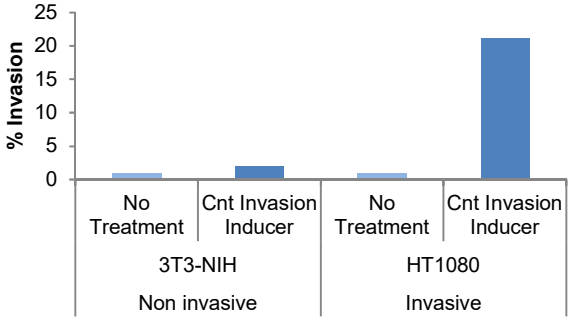


Figure 1: (A) Cell Invasion plate: The cells are added to the Top Chamber and the Control Invasion Inducer or chemoattractants are added to the Bottom Chamber. **(B) Cell Invasion:** 3T3-NIH and HT-1080 cells were starved overnight and treated with Control Invasion Inducer or remain untreated (No Treatment). Treatment with Control Invasion Inducer demonstrated a significant increase in invasion of HT 1080 cells as compare to 3T3-NIH control cells **(C) 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.

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