Cell Invasion Assay Kit (Laminin), 96-well, 8 µm (#BN01088)

(Catalog # BN01088; 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 Laminin, 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. Applications:

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

III. Sample Type:

- Invasive cell lines
- Invasion inhibitor or stimuli

IV. Kit Contents:

Components	BN01088	Cap Code	Part number
Wash Buffer	2 x 100 ml	NM	BN01088-1
Cell Dissociation Solution	10 ml	NM	BN01088-2
Control Invasion Inducer	1.5 ml	Red	BN01088-3
Cell Dye	1 ml	Blue	BN01088-4
Cell Invasion Chamber	1 each	Plate	BN01088-5
Laminin	4 x Vial	Green	BN01088-6

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 assay.

- **Wash Buffer, Cell Dissociation Solution and Control Invasion Inducer:** Store at -20°C. Bring to 37°C before use. Stable for six months.
- **Cell Dye:** Aliquot and store at -20°C. Bring to 37°C before use.
- **Cell Invasion Chamber:** Open under sterile conditions. Keep at room temperature.
- **Laminin:** Add 50 µl of Wash Buffer to the Laminin under sterile hood. Resuspend using pipette. Aliquot under hood and store at -80°C, if needed.

VII. Cell Invasion Assay Protocol:

1. Dilute Laminin 1:20 using Wash Buffer. Add 40 µl of Laminin to coat desired wells of the Top Chamber. Incubate plate at room temperature for 2-3 hrs in flow hood or overnight 2-8°C to gel the Laminin. Check the chamber from the side to make sure the plates are dried. Incubate for a longer duration if needed.
2. Grow enough cells to perform a Cell Invasion Assay and a Standard Curve in desired media and culture conditions. Adherent cells should be cultured to ~80% confluence.
3. 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 cells and centrifuge at 1,000 x g, for 5 min. to pellet them. Resuspend the 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.
4. Under sterile conditions, disassemble the Cell Invasion Chamber and carefully remove the plate cover and the top chamber (Fig. A).

   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.

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

5. Standard Curve:

A. 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) in desired well(s) in a 96-well plate (white plate clear bottom). Serially dilute the cells 1:1 in Wash Buffer and generate a Standard Curve of cells (50,000, 25,000, 12,500, 6,250, 3,125, 1,562, 781, and 390) in 100 µl total volume. As blank, use 100 µl of Wash Buffer.

B. Add 10 µl of Cell Dye to each well. Incubate at 37°C for 1 hr. Read the fluorescence at Ex/Em = 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.

6. Separation of Invasive Cells:

A. 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.

B. 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.

C. Place the plate cover on top of bottom chamber and centrifuge the plate at 1,000 x g for 5 min. at room temperature.

D. Carefully aspirate the media from the bottom chamber, and wash the chamber with 200 µl Wash Buffer.

E. Centrifuge the plate at 1,000 x g for 5 min. at room temperature and aspirate the Wash Buffer from the bottom chamber.

7. Count Invasive Cells:

A. For every eleven wells to be assayed, prepare a mix of 100 µl of Cell Dye in 1 ml of Cell Dissociation Solution. Mix well. Make desired amount of Cell Dye solution depending on the number of wells.

B. 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*. After incubation, disassemble the Cell Invasion Chamber and remove the top chamber.

C. Read the plate at Ex/Em = 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:

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

***Note:** During incubation with Cell Dissociation Solution/Cell 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.**

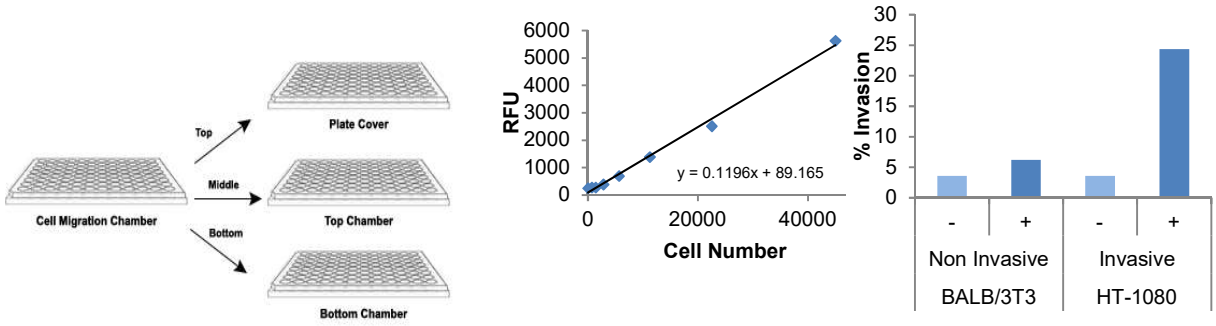

Figure 1: (a) Cell Invasion plate: The cells are added to the Top Chamber and the Control Invasion Inducer or chemoattractant are added to the Bottom Chamber. **(b) Standard Curve:** HT-1080 cells were harvested, counted and serially diluted to obtain desired cell number. Cells were incubated according to the protocol. **(c) Cell Invasion:** NIH-3T3 and HT-1080 cells were starved overnight and treated with Control (Cnt) 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 NIH-3T3 control cells.

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