Cell Invasion Assay Kit (Fibronectin), 96-well, 8 µm (#BN01099)

(Catalog # BN01099; 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 and mechanical stimuli. During invasion, the 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 Fibronectin, a high-molecular weight extracellular matrix protein. The cells invade the fibronectin matrix and then migrate through a semipermeable membrane in the Boyden chamber in response to stimulants or inhibitory compounds. The percentage of cell invasion can be analyzed directly in a 96-well microplate 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 (*e.g.* HT-1080 human fibrosarcoma cells)
- Invasion inhibitor or invasion-inducing stimulus

IV. Kit Contents:

Components	BN01099	Cap Code	Part number
Wash Buffer	2 x 100 ml	NM	BN01099-1
Cell Dissociation Solution	15 ml	NM	BN01099-2
Control Invasion Inducer	1.5 ml	Red	BN01099-3
Cell Dye	1.5 ml	Blue	BN01099-4
Cell Invasion Chamber	1 each	Plate	BN01099-5
Human Fibronectin (1 mg/ml)	500 µl	Green	BN01099-6

V. User Supplied Reagents & Equipment:

- Fluorescence microplate reader
- Cell culture media and humidified CO_2 cell culture incubator
- Sterile cotton swabs
- Clear 96-well plates with flat bottom

VI. Storage and Reagents Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Open all reagents and perform assay under sterile conditions (*e.g.* a cell culture-rated laminar flow hood) only. Read entire protocol before performing the assay.

- **Wash Buffer, Cell Dissociation Solution and Control Invasion Inducer:** Store at -20°C. Warm to 37°C before use and open under sterile conditions. Stable for six months.
- **Cell Dye:** Aliquot and store at -20°C, **protected from light**. Bring to 37°C before use.
- **Cell Invasion Chamber:** Open under sterile conditions. Keep at room temperature in a sterile environment.
- **Human Fibronectin (1 mg/ml):** Prior to use, thaw at 37°C. **Do not vortex or agitate Human Fibronectin solution.** Open under sterile conditions and aliquot/store remaining solution at +4°C. Avoid additional freeze/thaw cycles. Stable for six months at +4°C.

VII. Cell Invasion Assay Protocol:

1. Top Chamber Membrane Fibronectin Coating:

a. Dilute Human Fibronectin stock solution at a 1:5 ratio using Wash Buffer (if coating the entire assay plate, mix 500 µl Human Fibronectin stock with 2 ml Wash Buffer). Add 25 µl of the diluted solution to each of the desired wells of the top chamber to coat. Incubate plate at room temperature in a sterile environment (cell culture laminar flow hood) until fibronectin has dried, forming a thin film (usually about 2-3 hours, check the chamber from the side to ensure wells are dried, incubate for a longer time if needed).

b. Rinse the fibronectin-coated wells once with 50 µl of Wash Buffer. Gently aspirate buffer (taking care not to scratch the coated surface or top chamber membrane) and allow plate to dry in a sterile environment.

Note: Once coated with fibronectin, the top chamber may be stored at +4°C in a closed container for up to 2 weeks.

2. Grow enough cells to perform a Cell Invasion Assay and a Standard Curve in desired culture media and conditions. Adherent cells should be cultured to ~80% confluence.

3. Prior to the assay, starve cells for 18-24 hours in serum-free medium (0.5% serum can be used, if needed). After serum starvation, harvest cells and centrifuge at 1000 x *g* for 5 min to pellet them. Resuspend the cell pellet in Wash Buffer and count the number of cells using a hemocytometer or automated cell counter. Resuspend cells at a density of 1 x 10^6 cells/ml in serum-free medium.

4. Under sterile conditions, dissemble 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. To prepare invasion Positive Control well(s), 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 (~50000 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 culture medium. Carefully replace the plate cover and incubate the Cell Invasion Chamber at 37°C in a humidified CO_2 incubator for 2-72 hrs.

Note: Invasive cells pass through the fibronectin matrix and semipermeable membrane and cling to the outer side of the top chamber. Non-invasive cells stay in the top chamber. Incubation time required for invasion will vary by cell line and chemoattractant type.

5. Standard Curve Preparation:

a. For each cell type, prepare a standard curve by adding a 100 µl cell suspension (1 x 10^6 cells/ml, ~100000 cells) to a well in a clear 96-well plate (not included). Add 50 µl Wash Buffer to a series of six additional adjacent wells. Serially dilute the cells 1:1 in Wash Buffer by sequentially transferring 50 µl and mixing by pipetting up and down until reaching the last well, yielding a standard curve of 2-fold serially-diluted cells (50000, 25000, 12500, 6250, 3125, 1562 and 781 cells/well). Bring the total volume of all standard curve wells to 100 µl with Wash Buffer. As a background control (0 cells/well blank), add 100 µl of Wash Buffer to an empty well.

b. Prepare a sufficient amount of Cell Dye working solution by diluting the Cell Dye at a 1:5 ratio in Wash Buffer (for each well, mix 10 µl Cell Dye with 40 µl Wash Buffer). Add 50 µl of diluted Cell Dye to each well (including the 0 cell/well blank). Incubate at 37°C for 1 hour. Read the fluorescence of all standard wells at Ex/Em = 535/587 nm. Plot the standard curve (Number of Cells/Well vs RFU Value obtained) and fit the data points using a linear regression. The equation for the trend line is used for analysis of invasion data.

Note: Each cell type requires a separate standard curve. The Cell Invasion RFU reading should fall within the linear range of the standard curve. We recommend using duplicate or triplicate wells for the standard curve to minimize well-to-well variance.

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 top chamber membrane or fibronectin 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 1000 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 1000 x *g* for 5 min at room temperature and aspirate the Wash Buffer from the bottom chamber.

7. Count Invasive Cells:

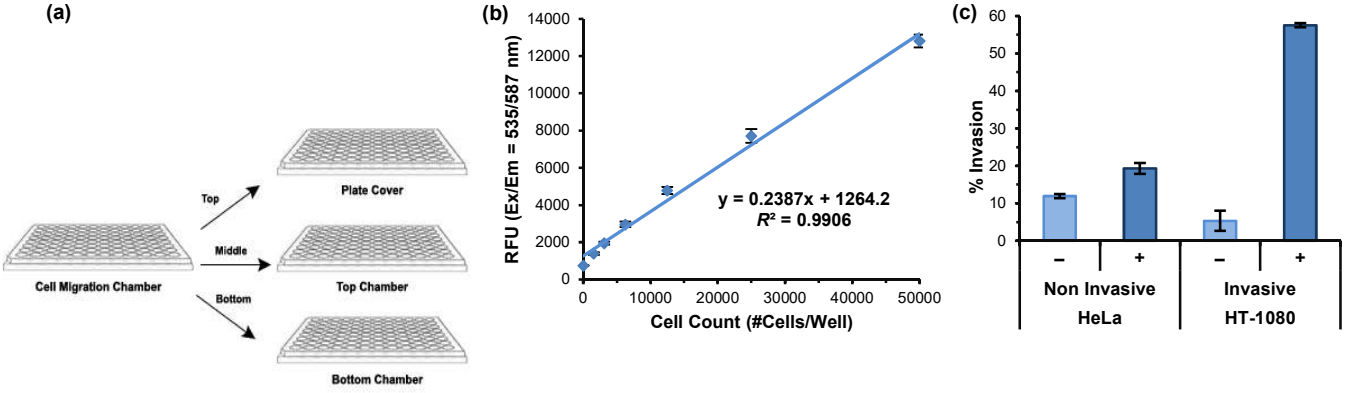
a. For every well to be assayed, prepare 150 µl of Cell Dissociation/Dye solution by combining 10 µl of Cell Dye and 140 µl of Cell Dissociation Solution. Make enough of the Cell Dissociation/Dye solution as needed, depending on the number of wells.

b. Add 150 µl of the Cell Dissociation/Dye 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 a CO_2 cell-culture incubator for 60 min. After incubation, disassemble the Cell Invasion Chamber and remove the top chamber.

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 membrane.

c. Read the fluorescence of the bottom chamber wells at Ex/Em = 535/587 nm. Calculate the number of cells that have invaded using the equation of the regression line obtained from standard curve. Percentage Invasion can be calculated as follows:

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



Added to the Top Chamber and the Control Invasion Inducer or other chemoattractant is added to the Bottom Chamber. Invasive cells will pass through the fibronectin matrix and Top Chamber membrane. **(b) Standard Curve:** HT-1080 cells were harvested, counted and serially diluted to obtain desired cell number. Cells were incubated and treated with cell viability dye according to the protocol. **(c) Cell Invasion:** HeLa and HT-1080 cells were serum starved overnight and treated with Control Invasion Inducer (+) or remained untreated (–). Treatment with Control Invasion Inducer resulted in a significant increase in invasion of HT-1080 cells across the membrane as compared to HeLa control cells.

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