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EZCell™ Cell Invasion Assay Kit (Collagen IV), 8 µm

Rev 07/19

(Catalog # K919-12; 12 inserts; 12 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. BioVision's Cell Invasion Assay Kit utilizes a Boyden chamber coated with Collagen IV, 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	K919-12	Cap Code	Part number
Wash Buffer	25 ml	WM	K919-12-1
Cell Dissociation Solution	6 ml	NM	K919-12-2
Control Invasion Inducer	300 µl	Red	K919-12-3
Cell Dye	1 vial	Green	K919-12-4
Cell Invasion Chamber	1 each	Plate	K919-12-5
Collagen IV	1.1 ml	NM	K919-12-6

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:** Add 100 µl of DMSO (Not provided) to the vial to dissolve the dye. Aliquot and store at -20°C.
- **Cell Invasion Chamber:** Open under sterile conditions. Keep at room temperature.
- **Collagen IV:** Aliquot under hood and store at -20°C, if needed.

VII. Cell Invasion Assay Protocol:

1. Add 100 µl Collagen IV 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 form a thin film of Collagen IV. Check the chamber from the side to make sure the plates are dried. Incubate for a longer duration if needed. Wash the plate coated with collagen IV three times with 1M Tris-Cl (not provided).
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 600 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 60 µl of Control Invasion Inducer to 540 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 200 µl (2-3 x 10^5 cells) of cell suspension to each well of the top chamber. Add desired stimulator or inhibitor to the top well, and gently mix. 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 Collagen IV 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 50 µl volume. As blank, use 100 µl of Wash Buffer.

B. Dilute Cell Dye 1:250 in PBS and add 50 µl of diluted Cell Dye to each well. Incubate at 37°C for 30 min. Read the fluorescence at Ex/Em = 485/530 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. Add 50 µl of Cell Dye (1:250 diluted) in 450 µl of Cell Dissociation Solution. Mix well. Make the Cell Dye solution as desired depending on the number of wells.

B. Add 500 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 CO2 incubator for 30 min*.

C. After incubation, disassemble the Cell Invasion Chamber and remove the top chamber and transfer 100 µl of mix from the bottom chamber to the 96-well white plate (the same plate having Standards).

D. Read the plate at Ex/Em = 485/530 nm. Multiply the reading by 5 to account for the 5X higher volume in each well of the 24-well plate. 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.**

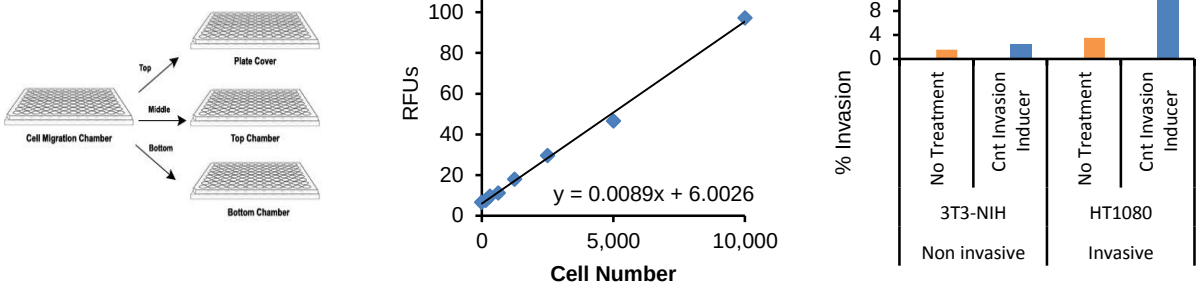

Figure 1: (a) Cell Invasion plate: The cells are added to the Top Chamber and the Control Invasion Inducer or chemoattractant are added to the Bottom Chamber. **(b) Standard Curve:** HT-1080 cells were harvested, counted and serially diluted to obtain desired cell number. Cells were incubated according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

VIII. RELATED PRODUCTS:

EZCell™ Invasion Assay (Basement Membrane), 96-well, 8 µm (K912)
EZCell™ Invasion Assay (Laminin), 96 and 24-well, 8 µm (K914, K915)
EZCell™ Invasion Assay (Collagen I), 96 and 24-well, 8 µm (K916, K917)
EZViable™ Calcein AM Cell Viability Assay Kit (K305)
EZCell™ Invasion Assay (Collagen IV), 96-well, 8 µm (K918)

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