Cell Migration/Chemotaxis Assay Kit (24-well, 5 µm) (BN01084)

(Catalog BN01084; 12 assays; Store at -20°C)

I. Introduction:

Cell migration is a process by which cells move from one location to another. Cell motility is observed in unicellular organisms, and is essential for the development and maintenance of multicellular organisms. Cells often migrate in response to specific external stimuli, including chemical & mechanical signals. Errors during this process have serious consequences, including intellectual disability, vascular disease, tumor formation and metastasis. Assay Genie's Cell Migration/Chemotaxis Assay Kit utilizes a Boyden chamber, where the cells migrate through a semipermeable membrane under different stimuli. Cell migration can be analyzed directly by reading fluorescence (Ex/Em = 530/590 nm) in a plate reader. Our assay is easy to use, sensitive and adaptable to high-throughput systems.

II. Application:

- Measure cell migration in response to stimuli
- Screen, study or characterize compounds that influence chemotaxis/cell migration

III. Sample Type:

- Adherent and suspension cells

IV. Kit Contents:

Components	BN01084	Cap Code
Wash Buffer	100 ml	NM
Cell Dissociation Solution	14 ml	NM
Control Migration Inducer	1.5 ml	Red
Cell Dye	1 ml	Blue
Cell Migration Chamber	1 each	Plate

V. User Supplied Reagents & Equipment:

- Fluorescence Plate Reader
- Cell Culture Media
- Cotton Swabs
- Centrifuge to spin 24-well plate
- 96-well white plate with clear flat bottom

VI. Storage Conditions 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.

- **Cell Migration Chamber:** Open under sterile conditions.
- **Control Migration Inducer, Cell Dissociation Solution, and Wash Buffer:** Store at -20°C. Bring to 37°C before use. Stable for six months after the first thaw.
- **Cell Dye:** Aliquot and store at -20°C. Bring to 37°C before use.

VII. Cell Migration Assay Protocol:

1. Grow enough cells to perform a Cell Migration Assay and a Standard Curve in desired media and culture conditions. Adherent cells should be cultured to ~80% confluence. Prior to the assay, starve cells for 18-24 hrs in 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 serum-free media and count the number of cells using a hemocytometer or an automated cell counter. Resuspend cells at 1 x 10^6 cells/ml in a serum-free media. Under sterile conditions, disassemble the Cell Migration Chamber (Fig. 1) and carefully remove the plate cover and the top chamber.

Bottom Chamber: Add 600 µl of serum-free media 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 Migration Inducer to 540 µl of media in the bottom chamber. Place the top chamber back into the bottom chamber. Ensure no air bubbles are trapped between the top and the bottom chamber.

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 place the plate cover and incubate the Cell Migration Chamber at 37°C in CO_2 incubator for 2-48 hrs.

Notes:

a) Migratory cells pass through the polycarbonate membrane and/or cling to the outer side of the top chamber. Non-migratory cells stay in the upper chamber.

b) If required, media with 0.1% serum can be used in top chamber.

2. Standard Curve: Each cell type requires a specific Standard Curve. Prepare a Standard Curve by adding 50 µl cell suspension (1 x 10^6 cells/ml, 5 x 10^4 cells) per well in 96-well white plate with clear bottom. Serially dilute the cells 1:1 in Wash Buffer and generate a Standard Curve of cells (50000, 25000, 12500, 6250, 3125, 1562, 781 and 390) in 100 µl total volume. As blank, use 100 µl of Wash Buffer. 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 of Number of Cells Vs RFU obtained. Fit the data points using a linear trendline with zero intercept. The equation for the straight line and R-square value are used for data analysis of samples.

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

3. **Separation of Migrated Cells:** After desired incubation for cell migration, carefully remove the plate cover and aspirate media from the top chamber without puncturing the membrane. Remove cells from the top chamber carefully using a cotton swab. Disassemble the Cell Migration 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 and cells with 500 µl Wash Buffer. Centrifuge the plate at 1,000 x g for 5 min. at room temperature and aspirate the Wash Buffer from the bottom chamber.
4. **Count Migrated Cells:** Make the cell dye solution as desired depending on the number of wells. Add 100 µl Cell Dye to 1 ml Cell Dissociation Solution. Mix well. Add 550 µl of the mix to each well of bottom chamber. Reassemble the Cell Migration Chamber by placing the top chamber into the bottom chamber. Incubate at 37°C in CO_2 incubator for 60 min. Gently tap the plate on the side to dissociate migratory cells that cling to the outer side of the top chamber. Remove the top chamber, and transfer 110 µl of mix from the bottom chamber to a 96-well white plate with clear flat bottom. Read the plate at Ex/Em = 530/590 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 migrated using the equation of the straight line obtained from the Standard Curve.

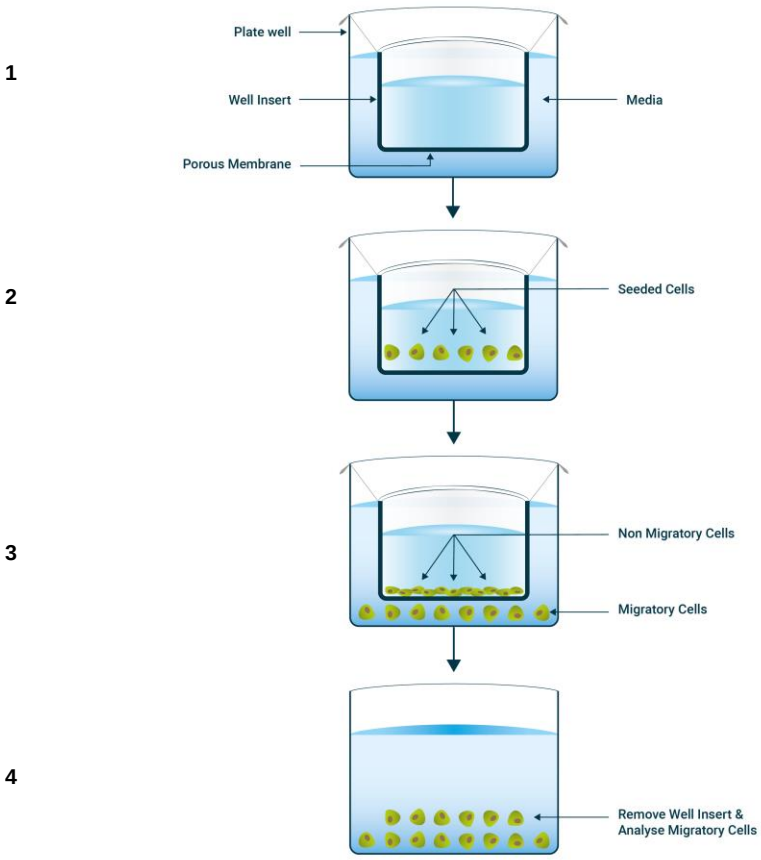

Figure 1: Assay Genie Cell Migration/Chemotaxis Kit: 1) A Boyden Chamber is used to investigate cell migration and chemotaxis. 2) Cells are added to the well insert and either control migration inducers or chemoattractants added to the bottom chamber. 3) Migratory cells move through the porous membrane while cells that do not migrate remain in the well insert. 4) The well insert is removed, and migratory cells are analyzed.

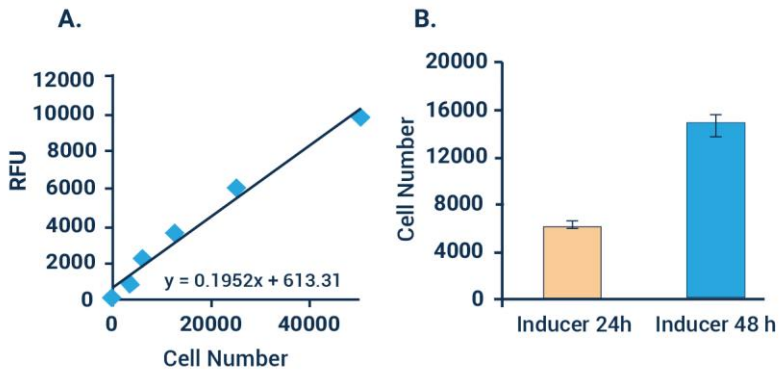

Figure 2: (A) Standard Curve: Monocytes/macrophage cells were harvested, counted, and serially diluted to obtain desired cell number. **(B) Cell Migration:** Monocytes/macrophage cells were starved overnight and treated with Control Migration Inducer for 24h, 48h or left untreated (control cells). Treatment with Control Migration Inducer demonstrated a significant increase in migration with time. Control reading was subtracted from inducer reading. Cells were incubated according to the protocol with Cell Dye and RFU (Ex/Em = 530/590 nm) was measured.