

# Cell Invasion Assay Kit

## (Fibronectin, 8 µm, 96-well)

### Instruction Manual

Catalog Number	PK-CA577-K925																							
Description	<p>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 &amp; 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.</p> <p>PromoKine's Cell Invasion Assay Kit (Fibronectin, 8 µm, 96-well) 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.</p>																							
Quantity	96 assays																							
Applications	<ul style="list-style-type: none"> <li>• Measure cell invasion in response to stimuli</li> <li>• Screen and characterize compounds that influence cell invasion</li> </ul>																							
Sample Type	<ul style="list-style-type: none"> <li>• Invasive cell lines (e.g. HT-1080 human fibrosarcoma cells)</li> <li>• Invasion inhibitor or invasion-inducing stimuli</li> </ul>																							
Kit Components	<table border="1"> <thead> <tr> <th>Components</th> <th>Quantity</th> <th>Cap code</th> </tr> </thead> <tbody> <tr> <td>Cell Invasion Chamber</td> <td>1 each</td> <td>Plate</td> </tr> <tr> <td>Human Fibronectin (1 mg/ml)</td> <td>0.5 ml</td> <td>Green</td> </tr> <tr> <td>Control Invasion Inducer</td> <td>1.5 ml</td> <td>Red</td> </tr> <tr> <td>Cell Dissociation Solution</td> <td>15 ml</td> <td>NM</td> </tr> <tr> <td>Wash Buffer</td> <td>2 x 100 ml</td> <td>NM</td> </tr> <tr> <td>Cell Dye</td> <td>1.5 ml</td> <td>Blue</td> </tr> </tbody> </table>	Components	Quantity	Cap code	Cell Invasion Chamber	1 each	Plate	Human Fibronectin (1 mg/ml)	0.5 ml	Green	Control Invasion Inducer	1.5 ml	Red	Cell Dissociation Solution	15 ml	NM	Wash Buffer	2 x 100 ml	NM	Cell Dye	1.5 ml	Blue		
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Storage & Stability	<p>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.</p> <p><b>Cell Invasion Chamber:</b> Open under sterile conditions. Keep at room temperature in a sterile environment.</p> <p><b>Human Fibronectin (1 mg/ml):</b> 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.</p> <p><b>Control Invasion Inducer, Cell Dissociation Solution and Wash Buffer:</b> Store at -20°C. Bring to 37°C before use. Stable for six months.</p> <p><b>Cell Dye:</b> Aliquot and store at -20°C. Bring to 37°C before use.</p>																							
User Supplied Reagents & Equipment	<ul style="list-style-type: none"> <li>• Fluorescence Plate Reader</li> <li>• Cell Culture Media and humidified CO2 cell culture incubator</li> <li>• Cotton Swabs</li> <li>• Centrifuge to spin 96-well plate</li> <li>• 96-well clear plate with flat bottom</li> </ul> <p>For human primary cells and the corresponding optimized cell culture media visit <a href="http://www.promocell.com">www.promocell.com</a>. For Cytokines/Chemokines and Growth Factors visit <a href="http://www.promokine.info">www.promokine.info</a>.</p>																							
Applications / Assay Protocol	<p><b>1. Top Chamber Membrane Fibronectin Coating:</b></p> <p>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</p>																							

dried, incubate for a longer time if needed).

b. Rinse the fibronectin-coated wells once with 50  $\mu$ l 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. Cell Culture:** 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. Assay Preparation:** 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 minutes 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  $1 \times 10^6$  cells/ml in serum-free medium.

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

Bottom Chamber: Add 200  $\mu$ l 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  $\mu$ l of Control Invasion Inducer to 180  $\mu$ l 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  $\mu$ l (~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  $\mu$ l with culture medium. Carefully replace the plate cover and incubate the Cell Invasion Chamber at 37°C in a humidified CO<sub>2</sub> incubator for 2-72 hours.

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  $\mu$ l cell suspension ( $1 \times 10^6$  cells/ml, ~100,000 cells) to a well in a clear 96-well plate (not included). Add 50  $\mu$ l Wash Buffer to a series of six additional adjacent wells. Serially dilute the cells 1:1 in Wash Buffer by sequentially transferring 50  $\mu$ l and mixing by pipetting up and down until reaching the last well, yielding a standard curve of 2-fold serially-diluted cells (50,000; 25,000; 12,500; 6250; 3125; 1562 and 781 cells/well). Bring the total volume of all standard curve wells to 100  $\mu$ l with Wash Buffer. As a background control (0 cells/well blank), add 100  $\mu$ l 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  $\mu$ l Cell Dye with 40  $\mu$ l Wash Buffer). Add 50  $\mu$ l 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 minutes at room temperature.

d. Carefully aspirate the media from the bottom chamber, and wash the chamber with 200  $\mu$ l Wash Buffer.

e. Centrifuge the plate at 1000 x g for 5 minutes 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  $\mu$ l of Cell Dissociation/Dye solution by combining

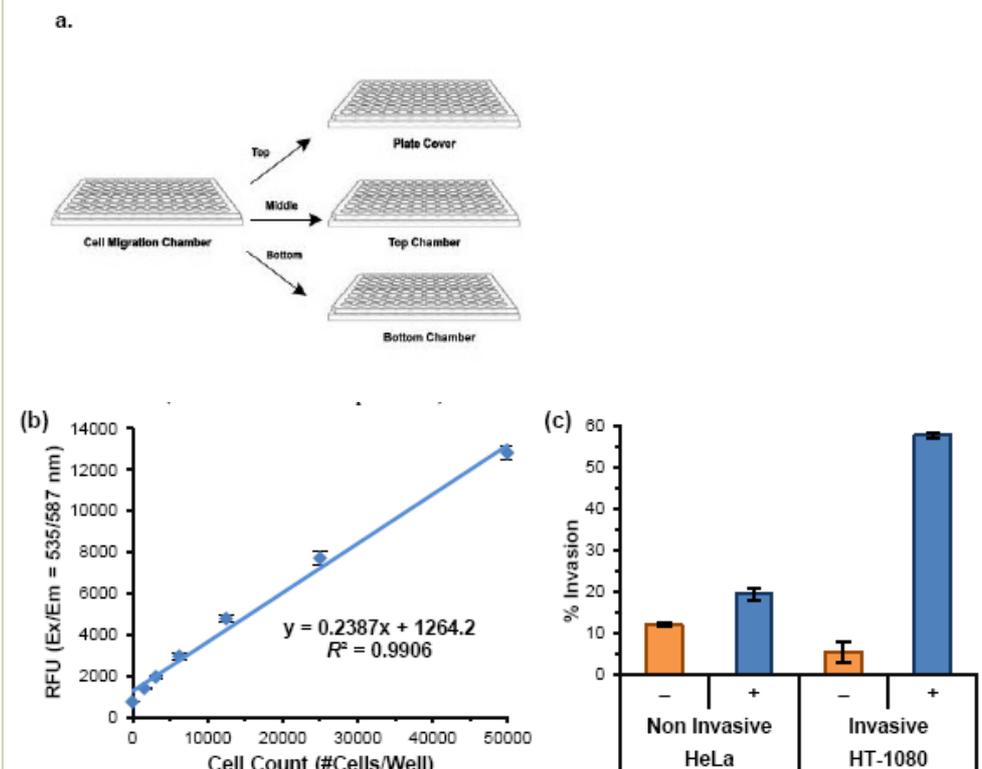
10 µl of Cell Dye and 140 µl of Cell Dissociation Solution. Make enough of the Cell Dissociation/Dye solution as needed, depending on the number of wells.

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<sub>2</sub> cell-culture incubator for 60 minutes. 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:

$$\% \text{ Invasion} = \frac{\text{Number of Cells in Lower Chamber}}{\text{Total Number of Cells added to Top Chamber}} \times 100$$



**Figure 1:** (a) Cell Invasion Plate: Cells are 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.

**Intended Use**

For in vitro research use only. Not for diagnostic or therapeutic procedures.

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