Version 4a, Last updated 23 August 2023

ab235885
Cell Invasion Assay Kit (Laminin), 24-well, 8 µm

For the measurement of cell migration in response to stimuli.

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

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1. Overview

Cell Invasion Assay Kit (Laminin), 24-well, 8 µm (ab235885) 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

Prepare cells.

Prior to the assay, starve cells for 18-24 hr in serum-free media.

Set up cell invasion assay containing desired chemoattractant in the bottom chamber. Incubate the Cell Chamber (12 x 8µm)/Cell Invasion Chamber at 37°C in CO_2 incubator for 2-48 hrs.

Prepare Standard Curve for each cell type.

Wash Cells.

Add Cell Dye I/Cell dye and incubate at 37°C in CO_2 incubator for 60 minutes.

Disassemble the Cell Chamber (12 x 8µm)/Cell Invasion Chamber, remove the top chamber and read the bottom well at Em/Ex = 530/590 nm.

2. Materials Supplied and Storage

Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Components are stable for 6 months after the first thaw.

Aliquot components in working volumes before storing at the recommended temperature.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Wash Buffer I/Wash Buffer	25 mL	-20°C	-20°C
Cell Dissociation Solution II/Cell Dissociation Solution	6 mL	-20°C	-20°C
Control Migration Inducer/Control Invasion Inducer	300 µL	-20°C	-20°C
Cell Dye I/Cell Dye	1.5 mL	-20°C	-20°C
Cell Chamber (12 x 8µm)/Cell Invasion Chamber	1	-20°C	-20°C
Laminin	1 Vial	-20°C	-80°C

3. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Fluorescence Plate Reader.
- Cotton Swab.
- Centrifuge to spin 96-well plate.
- 96-well clear bottom white plate.

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

5.1 Wash Buffer I/Wash Buffer

1. Ready to use as supplied.
2. Bring to 37°C before use.
3. Stable for six months after the first thaw.

5.2 Cell Dissociation Solution II/Cell Dissociation Solution

1. Ready to use as supplied.
2. Bring to 37°C before use.
3. Stable for six months after the first thaw.

5.3 Control Migration Inducer/Control Invasion Inducer

1. Ready to use as supplied.
2. Bring to 37°C before use.
3. Stable for six months after the first thaw.

5.4 Cell Dye I/Cell Dye

1. Ready to use as supplied.
2. Aliquot and store at -20°C.
3. Bring to 37°C before use.

5.5 Cell Chamber (12 x 8µm)/Cell Invasion Chamber

1. Open under sterile conditions.
2. Keep at room temperature.

5.6 Laminin

1. Add 50 µL of Wash Buffer I/Wash Buffer to the Laminin under a sterile hood. Resuspend using pipette.
2. Aliquot under hood and store at -80°C, if needed.
3. Dilute the Laminin solution 1:50 with Wash Buffer I/Wash Buffer prior to use.

6. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.
- Assay all standards, controls and samples in duplicate.

6.1 Cell Invasion Assay Protocol:

1. Add 100 µL of the diluted Laminin (step 5.6) to coat desired wells of the Top Chamber. Incubate plate at 37°C in an incubator for the Laminin to gel for 2-3 hours or overnight at 4°C.
2. Grow cells of interest in desired media and culture conditions. Grow enough cells to perform a Cell Invasion Assay and a Standard Curve.
3. Adherent cells should be cultured to ~80% confluence.
4. Prior to the assay, starve cells for 18-24 hours in a serum-free media (0.5% serum can be used, if needed).
5. After starvation, harvest the cells and centrifuge at 1,000 x *g*, for 5 minutes to pellet cells.
6. Resuspend cell pellet in Wash Buffer I/Wash Buffer and count the number of cells using hemocytometer or automated cell counter.
7. Resuspend cells at 1 x 10^6 cells/mL in a serum-free media.
8. Under sterile conditions, disassemble the Cell Chamber (12 x 8µm)/Cell Invasion Chamber and carefully remove the plate cover and the top chamber (figure 1).

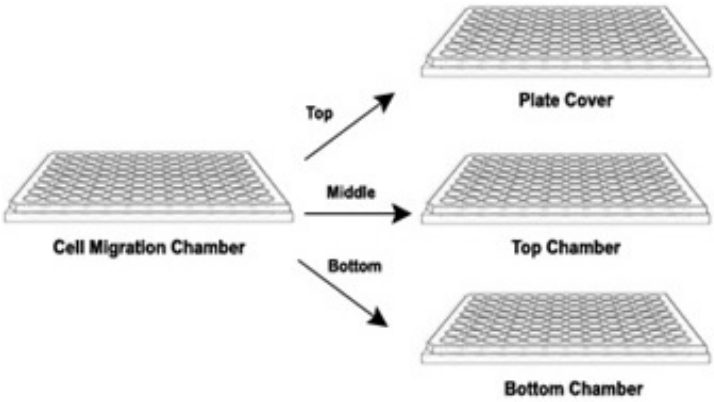

Figure 1. Cell Invasion plate: The cells are added to the Top Chamber and the Control Migration Inducer/Control Invasion Inducer or chemoattractant are added to the Bottom Chamber.

9. **Bottom Chamber:** Add 600 µL of medium per well containing desired chemoattractant to the bottom chamber.
10. In control well(s), we recommend omitting the chemoattractant.
11. For Positive Control, add 60 µL of Control Migration Inducer/Control Invasion Inducer to 540 µL of medium in the bottom chamber.
12. Reassemble the top and bottom chambers while ensuring no air bubbles are trapped between them
13. **Top Chamber:** Add 200 µL (2-2 x 10^5 cells) of cell suspension to each well of the top chamber.
14. Add desired stimulator or inhibitor to the top well, and gently mix.
15. Make up the volume to 100 µL with media. Carefully place the plate cover and incubate the Cell Migration Chamber at 37°C in CO_2 incubator for 2-48 hours.

6.2 Standard Curve:

1. 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) per well in a 96-well plate (white plate clear bottom).
2. Serially dilute the cells 1:1 in Wash Buffer I/Wash Buffer and generate a Standard Curve of cells (50,000, 25,000, 12,500, 6,250, 3,125, 1,562 and 781) in 100 µL total volume.
3. As blank, use 100 µL of Wash Buffer I/Wash Buffer.
4. Add 10 µL of Cell Dye I/Cell Dye to each well.
5. Incubate at 37°C for 1 hour.
6. Read the fluorescence at Ex/Em = 530/590 nm.
7. Plot the Standard Curve of Number of Cells Vs RFU obtained.
8. Fit the data points using a linear trendline with zero intercept.
9. The equation for the straight line and R-square value are used for data analysis of samples.

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

6.3 Data Collection and Analysis:

1. 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.
2. Remove cells from the top chamber using a cotton swab. Disassemble the Cell Chamber (12 x 8µm)/Cell Invasion Chamber by removing the top chamber.
3. 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 minutes at room temperature.
4. Carefully aspirate the media from the bottom chamber, and wash the chamber with 500 µL Wash Buffer I/Wash Buffer.
5. Centrifuge the plate at 1,000 x *g* for 5 minutes at room temperature and aspirate the media from the bottom chamber.
6. For every twenty wells to be assayed, prepare a mix of 1 mL of Cell Invasion Dye in 10 mL of Cell Dissociation Solution II/Cell Dissociation Solution. Mix well.

7. Add 550 µL of the mix to each well of the bottom chamber.
8. Reassemble the Cell Chamber (12 x 8µm)/Cell Invasion Chamber by placing the top chamber into the bottom chamber. Incubate at 37°C in CO_2 incubator for 60 minutes.
9. Disassemble the Cell Chamber (12 x 8µm)/Cell Invasion Chamber, remove the top chamber and transfer 110 µl of mix from the bottom chamber to the 96-well white plate (the same plate having Standards).
10. Read the plate at Em/Ex = 530/590 nm Multiply the reading by 5 to account for the 5X higher volume in each well of the 24-well plate.

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

    Δ Note: During incubation with Cell Dissociation Solution II/Cell Dissociation Solution/Cell Invasion 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.

7. Data Analysis

1. Calculate the number of cells invaded using the equation of the straight line obtained from Standard Curve.
2. Percentage Invasion can be calculated as follows:

$$\% \text{ Invasion} = \frac{\text{B\# Cells in Lower Chamber}}{\text{Total \# Cells added to Top Chamber}} * 100$$

8. FAQs / Troubleshooting

General troubleshooting points are found at www.abcam.com/assaykitguidelines.

9. Typical Data

Data provided **for demonstration purposes only.**

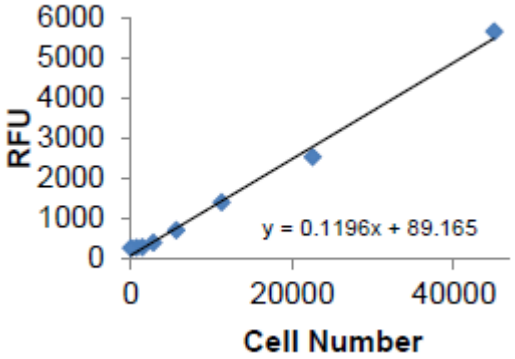

Figure 2. Standard Curve: HT-1080 cells were harvested, counted and serially diluted to obtain desired cell number. Cells were incubated according to the protocol with Cell Invasion Dye and fluorescence (Em/Ex 530/590) was measured.

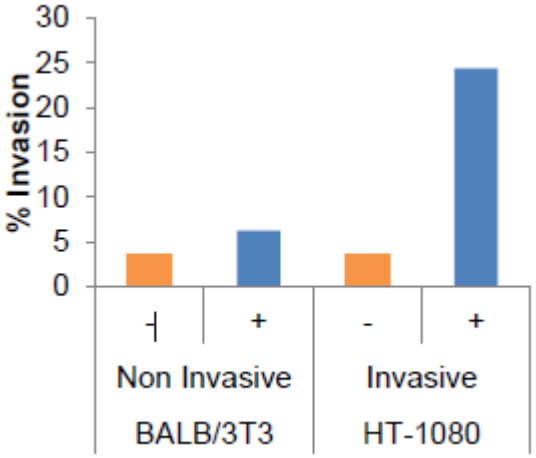

Figure 3. Cell Invasion: 3T3-NIH and HT-1080 cells were starved overnight and treated with Control Migration Inducer/Control Invasion Inducer or remain untreated (No Treatment). Treatment with Control Migration Inducer/Control Invasion Inducer demonstrated a significant increase in invasion of HT 1080 cells as compared to 3T3-NIH control cells.

10. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)