Version 2 Last updated 12 August 2019

ab235694 Cell Migration/Chemotaxis Assay Kit (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.

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1. Overview

Cell Migration/Chemotaxis Assay Kit (24-well, 8 µm) (ab235694) utilizes a Boyden chamber, where the cells migrate through a semi-permeable 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.

Prepare cells.

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

Set up cell migration assay containing desired chemoattractant in the bottom chamber. Incubate the Cell Migration Chamber at 37°C in CO_2 incubator for 2-48 h.

Prepare Standard Curve for each cell type.

Separate Migrated cells.

Add cell dye and count Migrated cells.

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	25 mL	-20°C	-20°C
Cell Dissociation Solution	6 mL	-20°C	-20°C
Control Migration Inducer	300 µL	-20°C	-20°C
Cell Dye	1.5 mL	-20°C	-20°C
Cell Migration Chamber	1	-20°C	-20°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 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

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

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

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

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

5.5 Cell Migration Chamber

1. Open under sterile conditions.

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 Migration Assay Protocol:

1. Grow enough cells to perform a Cell Migration Assay and a Standard Curve in desired media and culture conditions.
2. Adherent cells should be cultured to ~80% confluence. Prior to the assay, starve cells for 18-24 h in serum-free media (0.5% serum can be used if needed).
3. After starvation, harvest cells and centrifuge at 1,000 x *g*, for 5 minutes to pellet them.
4. Resuspend the cell pellet in serum-free media and count the number of cells using a hemocytometer or an automated cell counter.
5. Resuspend cells at 1 x 10^6 cells/mL in a serum-free media.
6. Under sterile conditions, disassemble the Cell Migration Chamber (Figure 1) and carefully remove the plate cover and the top chamber.

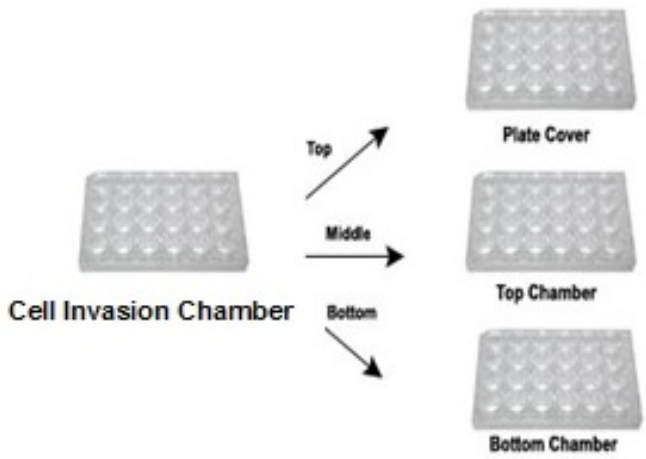

Figure 1. Cell Invasion plate: The cells are added to the Top Chamber and the Control Invasion Inducer or chemoattractant are added to the Bottom Chamber.

7. **Bottom Chamber:** Add 600 µL of serum-free media per well containing desired chemoattractant to the bottom chamber.
8. In control well(s), we recommend omitting the chemoattractant.
9. For positive control, add 60 µL of Control Migration Inducer to 540 µL of media in the bottom chamber.
10. Place the top chamber back into the bottom chamber.
11. Ensure no air bubbles are trapped between the top and the bottom chamber.
12. **Top Chamber:** Add 200 µL (2-3 x 10^5 cells) of cell suspension to each well of the top chamber.
13. Add desired stimulator or inhibitor to the top well, and gently mix.
14. Carefully place the plate cover and incubate the Cell Migration Chamber at 37°C in CO_2 incubator for 2-48 hours.

Δ Note: 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.

Δ Note: If required, media with 0.1% serum can be used in top chamber.

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 clear plate.
2. 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 100 µL total volume.
3. As blank, use 100 µL of Wash Buffer.
4. Add 10 µL of 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 Migration RFU reading should fall in the linear range of the Standard Curve. We recommend using triplicates for Standard Curve.

6.3 Separation of Invasive Cells:

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 Invasion Chamber by removing the top chamber. Invert the top chamber and set it aside.
3. 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.

5. Centrifuge the plate at 1,000 x g for 5 min. at room temperature and aspirate the Wash Buffer from the bottom chamber.

6.4 Count Invasive Cells:

1. For every twenty wells to be assayed, prepare a mix of 1 mL of Cell Dye in 10 mL of Cell Dissociation Solution. Mix well. Make the Cell Dye solution as desired depending on the number of wells.
2. Add 550 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 CO_2 incubator for 60 minutes.
3. After incubation, disassemble the 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).
4. 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.

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**.

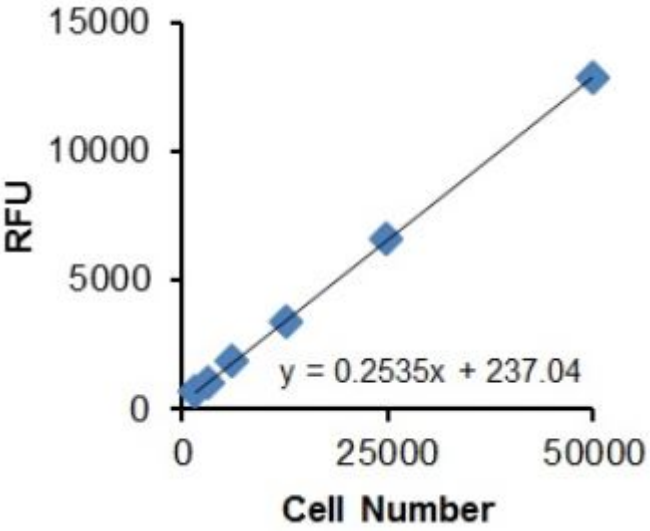

Figure 2. Standard Curve: HT-1080 cells were harvested, counted and serially diluted to obtain desired cell number. Cells were incubated according to the protocol.

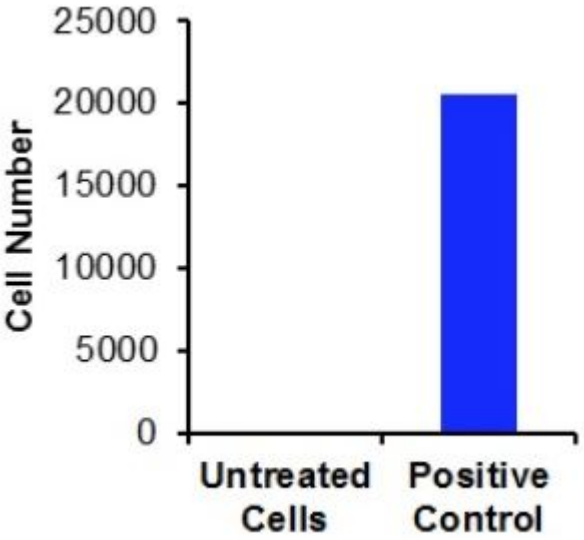

Figure 3. Cell Invasion: HT-1080 cells were starved overnight and treated with Control (Cnt) Invasion Inducer or remain untreated (Untreated cells). Treatment with Control Invasion Inducer demonstrated a significant increase in invasion as compared to untreated control cells.

10. Notes

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