Version 2c Last updated 25 March 2024

# ab235888 Cell Invasion Assay (Collagen IV), 96-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.

# Table of Contents

# 1. Overview

Cell Invasion Assay (Collagen IV), 96-well, 8 µm (ab235888) utilizes a Boyden chamber coated with Collagen IV, where the cells invade the matrix and then migrate through a semi-permeable 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 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 and incubate at 37°C in $CO_2$ incubator for 30 minutes.

Disassemble the Cell Invasion Chamber, remove the top chamber and read the bottom well at Em/Ex = 485/530 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 | 50 mL | -20°C | -20°C |
| Cell Dissociation Solution | 15 mL | -20°C | -20°C |
| Control Invasion Inducer | 300 µL | -20°C | -20°C |
| Cell Dye | 1 mL | -20°C | -20°C |
| Cell Invasion Chamber | 1 | -20°C | -20°C |
| Collagen IV | 5 mL | -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 clear bottom white plate.
- 1 M Tris-Cl pH 7.4

## 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 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

1. Add 100 µL of DMSO to the vial.
2. Aliquot and store at -20°C.
3. Bring to 37°C before use.

## 5.5 Cell Invasion Chamber

1. Open under sterile conditions.

## 5.6 Collagen IV

1. Aliquot under the hood and store at -20°C, if needed.

# 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 50 µL of Collagen IV to coat desired wells of the Top Chamber. Incubate plate at room temperature for 2-3 hours in flow hood or overnight at 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 coated plate three times with 100 µL 1 M Tris-Cl. Aspirate.
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 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 Invasion Chamber and carefully remove the plate cover and the top chamber.

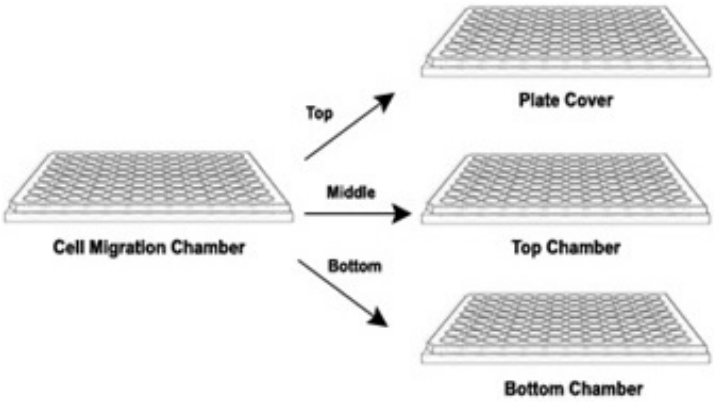


Figure 1. Cell Invasion plate: The cells are added to the Top Chamber and the Control Invasion Inducer or chemoattractant are added to the Bottom Chamber.

9. **Bottom Chamber:** Add 200 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 20 µL of Control Invasion Inducer to 180 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 50 µL (50,000 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 Invasion 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 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. Dilute Cell Dye 1:250 in PBS and add 50 µL of diluted Cell Dye to each well.
5. Incubate at 37°C for 30 minutes.
6. Read the fluorescence at Ex/Em = 485/530 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 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 200 µL 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. Dilute Cell Dye 1:500 in Cell Dissociation Solution. Mix well.
7. Add 100 µL of the mix to each well of the bottom chamber.

8. Reassemble the Cell Invasion Chamber by placing the top chamber into the bottom chamber. Incubate at 37°C in $CO_2$ incubator for 30 minutes.
9. Disassemble the Cell Invasion Chamber, remove the top chamber and read the bottom well at Em/Ex = 485/530 nm.

   **Δ 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.

   **Δ Note**: During incubation with 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**.

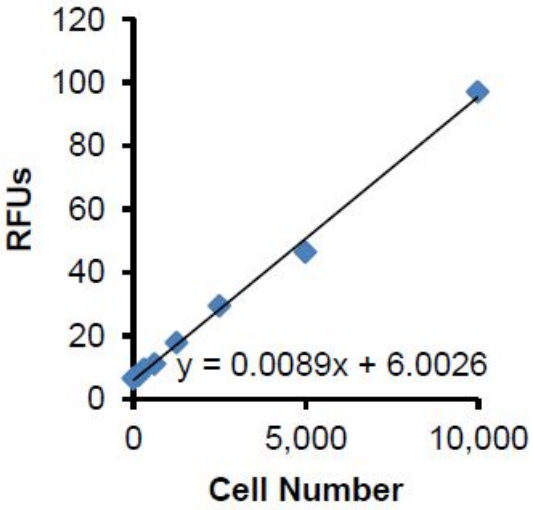


**Figure 2**. Standard Curve: HT-1080 cells were harvested, counted and serially diluted to obtain desired cell number. Cells were incubated according to the protocol.

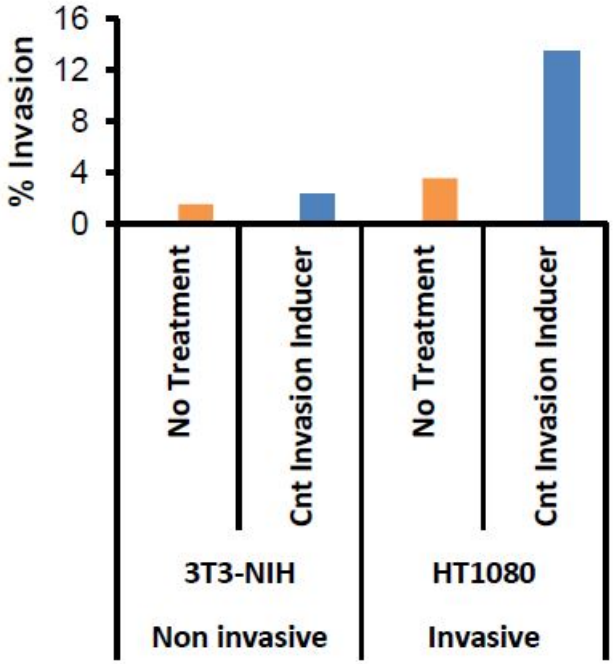


Figure 3. Cell Invasion: NIH-3T3 and HT-1080 cells were starved overnight and treated with Control (Cnt) Invasion Inducer or remain untreated (No Treatment). Treatment with Control Invasion Inducer demonstrated a significant increase in invasion of HT 1080 cells as compared to 3T3-NIH control cells.

# 10. Notes

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