Cell Migration, Chemotaxis and Invasion Assay Using Staining

Protocol

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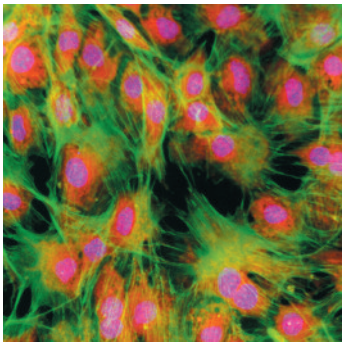
Introduction

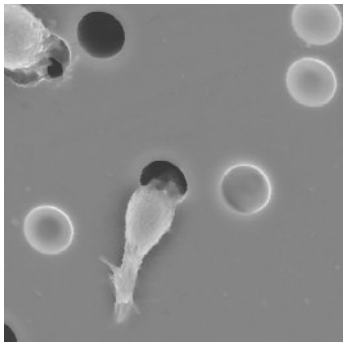
Cell migration – the movement of cells from one area to another in response to a chemical signal – is central to a variety of functions, such as wound repair, cell differentiation, embryonic development and the metastasis of tumors. Cell invasion, although similar to cell migration, requires a cell to migrate through an extracellular matrix (ECM) or basement membrane extract (BME) barrier by first enzymatically degrading that barrier and then establishing in a new location. Cell invasion is exhibited by both normal cells, e.g., response to inflammation and by tumor cells in the process of metastasis. Therefore, understanding the underlying mechanisms of this process are important for a wide array of biological systems.

The advent of disposable inserts, such as Transwell® permeable supports from Corning Life Sciences, provides a relatively simple *in vitro* approach to performing chemotaxis and cell invasion assays. Common barriers employed for invasion assays include collagen, fibronectin and laminin coatings as well as more complex extracellular or basement membrane extracts. More elaborate invasion assays establish a monolayer of endothelial cells on the permeable support in place of, or in addition to, the protein coatings or BME listed above. Similarly, cells that secrete a paracrine growth factor can be cultured in the receiver wells of the permeable support system to act as the source of chemoattractant in either simple chemotaxis assays or for more elaborate invasion assays.

The following protocol outlines the steps for conducting a cell migration assay with HT-1080 cells that are enumerated by staining and microscopy. This is a generalized protocol and should be adapted to suit individual needs. This protocol utilizes Transwell permeable supports fom Corning, however, tables are provided with the proper volumes and amounts of pertinent materials and reagents to scale the assay for use with larger or smaller permeable supports.

For more information on optimizing chemotaxis or invasion assay, please refer to *Considerations When Optimizing your Chemotaxis or Invasion Assay with Corning Transwell Permeable Supports* (CLS-AN-188).





Materials

Cell Line

- HT-1080 Cells (Human Fibrosarcoma ATCC No. CCL-121)

Assay Plates

- 5.0 or 8.0 µm Transwell® Inserts (see end of protocol for part numbers)

Reagents

- Cell Dissociation Solution for harvesting cells (Life Technologies Cat. No. 13150-016)
- IMDM Medium with 10% FBS (Corning® cellgro® Cat. No. 10-016-CM and 35-010-CV, respectively)
- Serum-free Medium (SFM), IMDM medium without serum containing 1x ITS (Corning cellgro Cat. No. 25-800-CR)
- Dulbecco's Phosphate Buffered Saline (DPBS) with calcium and magnesium (Corning cellgro Cat. No. 21-030-CM)
- Sterile Deionized Water (Corning cellgro Cat. No. 25-055-CV)
- Crystal Violet Stain (Fisher Cat. No. 23-750-025)
- Cotton Swabs (Fisher Cat. No. 23-400-101)

Instruments

- 37°C CO_2 incubator
- Laminar flow hood
- Light microscope

Procedure

Grow enough cells in advance to accommodate the different cell concentrations required to set up assay (Table 2).

Note:
Read entire protocol before beginning assay.

Protocol Overview

Day 1 – Starve cells and coat Transwell inserts if necessary.

Day 2 – Plate cells in Transwell inserts and stimulate with FBS attractant.

Day 3 – Stain cells that pass through membrane.

Assay Preparation

Note:
Using the cell requirements from Table 2, prepare enough cell cultures one day prior to accommodate the number of cells required to set up the assay.

1. *Cell Maintenance*
   a. For this step, cultures should be below 80% confluence to ensure cells are properly starved without becoming confluent during the 24 hr starvation period.
   b. Remove serum-containing medium from cultures.
   c. Thoroughly but gently rinse cultures with PBS to remove all serum.
   d. Replace culture medium with serum-free IMDM medium (SFM). Return cultures to incubator for 24 hours.

Note:
To properly assess cell invasion, it is necessary to set up a few wells without an ECM barrier to determine the percent of cells that exhibit chemotaxis when protein degradation is not required.

2. *Transwell Coating*
   The concentration of barrier necessary for an invasion assay is dependent on the cell line, chemoattractant, and barrier being used. Corning recommends testing various concentrations to find the optimal concentration for an assay. The optimal concentration will provide the biggest difference between the invasive and noninvasive cell lines in response to a chemoattractant. For more information on coating Transwells please refer to *Considerations When Optimizing Coating Protocols for Corning Transwell Permeable Supports* (CLS-AN-134) and *Considerations when Optimizing your Chemotaxis or Invasion Assay with Corning Transwell Permeable Supports* (CLS-AN-188).
   a. Under sterile conditions prepare coating solution to desired concentration.
   b. Coat Transwell inserts with coating solution (See Note at left, and Table below)
   c. Incubate plate at room temperature or in an incubator at 37°C for desired coating time.

Table 1. Recommended Coating Volumes

Assay Plate Format	Number of Inserts	Insert Bottom Area (cm^2)	Coating/Insert (mL)	Total Coating/Plate (mL)
HTS Transwell-96	96	0.143	0.050	5
24 well	12*	0.3	0.100	2
12 well	12	1.1	0.3	4
6 well	6	4.5	1	6.5

*Costar® Transwell® 6.5 mm diameter inserts are packed 12 per 24 well plate.

Note:

The use of dissociation solution in place of trypsin with HT-1080 cells in this protocol resulted in a higher migratory response and lower variability. Consider experimenting with a variety of dissociation agents and methods for enhanced protocol optimization.

Plating Cells

3. *Harvest cells using a Cell Dissociation Solution or other suitable cell dissociation solution (see note).*
   a. Dilute dissociation solution with serum-free media or serum-free trypsin inhibitor.
   b. Spin cells down to remove cell dissociation solution and re-suspend in serum-free medium.
   c. Determine cell concentration and dilute cell suspension to necessary seeding concentration with serum free medium (Table 2).
   d. Aspirate excess coating solution from inserts if running invasion assay and plate cells.
   e. To a few receiver wells, add serum-free medium (no chemoattractant) as backaground controls. To the remaining receiver wells, add medium with serum (plus chemoattractant).
   f. Incubate cultures for 4 to 24 hours depending on how migratory or invasive the cell line is.

***Note:**

Cell seeding densities have been optimized for this protocol using HT-1080 cells. For best results, Corning recommends optimizing cell seeding densities for each cell line and given condition. It is important that there are enough cells to count once stained but not too many as to hinder the ability to accurately enumerate.

Table 2. Recommended Cell Seeding Concentrations and Volumes

Assay Plate Format	Cells/Well (x 10^5)*	Seeding volume/ Insert (mL)	Reservoir Volume (mL/Well)
HTS Transwell-96	0.2-0.5	0.05	0.150
24 well	0.5-1.0	0.100	0.65
12 well	1.0 to 2.0	0.385	1.0
6 well	4.0 to 6.0	1.5	2.0

Staining Migrated/Invaded Cells

4. *Prior to staining, non-migrated cells must be removed from the inserts manually in order to prevent fixation and staining to the membrane.*
   a. Aspirate medium from assay/receiver plate and Transwell inserts.
   b. Gently swab the inside of each insert using cotton swabs, taking care not to damage the membrane or touch the underside of the inserts.
   c. Wash apical inserts and receiver wells with wash buffer (Table 3).
   d. Add crystal violet stain solution to each insert and allow to sit for 10 minutes (Table 3).
   e. After the cells have been stained, thoroughly rinse the inserts with water until the water runs clear.
   f. Allow the inserts to dry completely before visualizing with a microscope.

***Note:**

Cells that pass through the membrane attach either to the bottom of the membrane or fall to the receiver plate. Generally, the loss of cells to the receiver plate is negligible. If the cell loss to the receiver plate appears significant, however, decrease the migration time of the experiment.

Table 3. Wash Buffer and Crystal Violet Stain Volumes

Assay Plate Format	Inserts (mL/Insert)	Receiver Plate (mL/Well)
HTS Transwell-96	0.1	0.02
24 well	0.2	0.4
12 well	0.5	1.0
6 well	1.0	2.0

Counting Migrated/Invaded Cells

5. Using a light microscope enumerate the number of stained cells in random fields within each Transwell® Insert (Figures 1 and 2).

Note:

Counts from multiple fields within a well should be averaged in order to calculate percent migration/ invasion. It is also good practice to sample the middle and perimeters of the insert to ensure more consistent results.

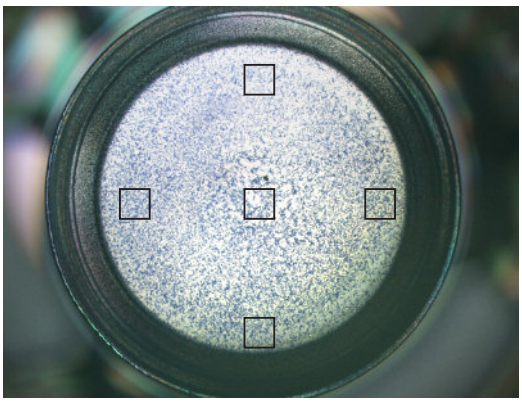


Figure 1. Example of 5 random fields to be counted.

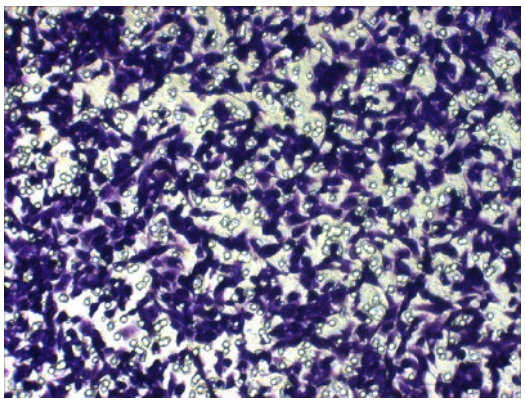


Figure 2. Migrated HT-1080 cells fixed and stained to 8.0 µm PET (Corning Cat. No 3464) 200x.

Calculating Percent Migration/Invasion

6. To calculate migration/invasion, the total number of cells per insert must be determined.

a. Count and average the total number of cells in each of the random fields within an insert.
Example: 203, 210, 220, 206, and 213 = 210

b. Divide this number by the area of the microscope viewing field and then multiply this number by the entire area of the Transwell insert.
Example: (210 / 0.001 cm^2) x 0.33 cm^2 = 69,300 cells

7. Once the total number of cells per insert is determined percent migration/invasion can be calculated by dividing this number by the number of cells seeded. Multiple this value by 100 to get a percent.

Example: (69,300/100,000) x 100 = 69.3% migration or invasion

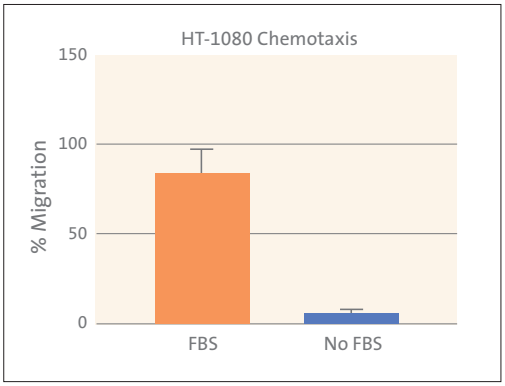


Figure 3. HT-1080 Migration After 4 Hours

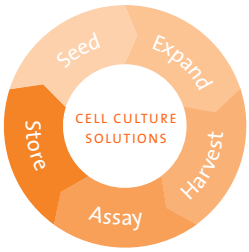
8. Data Interpretation

a. There should be little or no migration/movement without chemoattractant (serum).

b. The difference in percent invasion should be 20% or more between coating (cell invasion) and no coating (cell migration) when using FBS as a chemoattractant.

Transwell® Permeable Support inserts for Migration Assays

Cat. No.	Pore size (µm)	Format (mm)	Membrane Type	Notes
3415	3.0	6.5	PC	
3472	3.0	6.5	PET	
3421	5.0	6.5	PC	
3422	8.0	6.5	PC	
3458	8.0	6.5	PC	Pre-coated with Cultrex® BME
3464	8.0	6.5	PET	New
3402	3.0	12	PC	
3462	3.0	12	PET	
3414	3.0	24	PC	
3428	8.0	24	PC	
3452	3.0	24	PET	
3398/3399	3.0	24 HTS	PC	
3385/3386	3.0	96 HTS	PC	
3387/3388	5.0	96 HTS	PC	
3374/3384	8.0	96 HTS	PET	



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