# Considerations when Optimizing your Chemotaxis or Invasion Assay with Corning® Transwell® Permeable Supports

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## Application Note

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### Introduction

Chemotaxis and invasion assays are instrumental in studying wound repair, cell differentiation, cellular communication, embryonic development and tumor metastasis. Due to the complexity of these biological systems, optimization is required to set up well organized and reproducible studies. Although information is readily available with respect to known chemoattractants, cell seeding concentrations and incubation times, not much is known regarding specific model systems. The following information will highlight important factors to consider in order to optimize and obtain accurate results with chemotaxis and invasion assays using permeable supports.

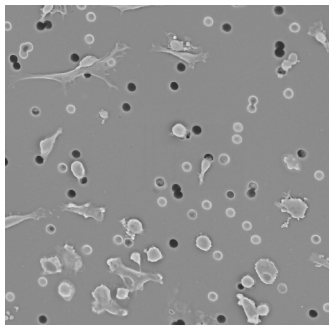
### Selecting the Right Transwell Permeable Support System

One of the most critical factors when performing chemotaxis and invasion assays is the proper selection of the Transwell Permeable Support System to be used. This decision hinges on two considerations, what pore size will give the optimal results and which format will be the most appropriate for the testing. The pore size will be dependent on the migratory cells being used. If the pore size is too small there will be little to no migration due to the fact that the cells simply cannot alter their shape enough to migrate through the pores. If, on the other hand, the pores are too large, the cells will be more likely to fall through the pores generating inaccurate migration assay results. As a reference starting point, 3.0 µm pores are recommended for small cells like leukocytes and 5.0 µm or 8.0 µm pores for larger endothelial and epithelial cells.

When selecting the ideal Transwell system it is important to consider how many wells, including replicates and controls, will be necessary to obtain quality data. Running each sample in triplicate minimally is recommended. Proper controls are also essential to understanding and validating the results. In a chemotaxis assay, some of these controls may include wells without chemoattractant (negative control) and wells with known chemoattractant (positive control). It may also be helpful to include some wells with a less migratory cell line in the presence of chemoattractant (e.g., MCF7 to FBS). When performing an invasion assay, include a control without the extracellular matrix protein barrier to evaluate the difference between migrating and invading cells. Additionally, it may be important to include a noninvasive line (e.g., NIH3T3) to confirm that the barrier being used is indeed inhibiting invasion. Understanding the desired controls and samples needed will aid in deciding what Transwell plate format to use (e.g., 24 well Transwell inserts or HTS Transwell-96 microplate).

### Enumerating Migrated Cells

Choosing an appropriate method to quantitate the number of migrating/invading cells is an important part of a chemotaxis assay. The method chosen will depend on many factors including equipment availability, reagent cost, cell type, as well as throughput. If the cells are nonadherent, they can simply be collected in the receiver microplate and enumerated by a number of counting methods such as a hemocytometer, flow cytometry, or some type of vital dye assay (e.g., MTT, fluorescent stain). If cells are adherent, an often used inexpensive method of enumeration involves gently swabbing the top of the permeable support membrane to remove nonmigrated cells, then fixing and staining the migrated cells to the underside of the membrane. The cells fixed and stained on the membrane can then be counted using a microscope to determine percent migration or invasion (Figure 1). If using this method of enumeration, it is important to count enough fields to get an accurate representation of cell number since sometimes cells migrate preferentially towards the center or edge of the insert. Other common methods of adherent cell enumeration involve dissociating or lysing cells from the membrane and using various reagents or assay kits to quantify migrated cells (e.g., Calcein AM). Although the second method of cell enumeration can be more expensive, it does have the advantages of being faster, allowing for higher throughput and reduced variability.



SEM photograph of HT1080 cells 500X magnification.

## Optimizing Seeding Concentration

Using the optimal seeding concentration is another important factor for migration assays to enable maximal signal compared to background. If too few cells are used, there will not be enough cells to accurately count or enumerate by assay due to the small sample size. Conversely, if too many cells are used, oversaturation of pores, counting fields, or assay signal can lead to inaccurate results. Run a titration of the cells with a known chemoattractant to determine what the optimal seeding concentration is for the assay and method of enumeration (Figure 2).

## Optimizing Chemoattractant Concentration

For every migration or invasion assay, the chemoattractant is critical for the success of the experiment. Therefore, when deciding which chemoattractant to use, it may be beneficial to examine multiple concentrations. Perform a serial dilution of the chemoattractant to select the optimal concentration. Moreover, if the option is available, it may be beneficial to assess multiple chemoattractants to ensure the best one for the assay is selected (Figure 3).

## Migration/Invasion Time

If using a well-studied model, the migration time may already be documented, otherwise, optimization should be performed. There are many factors that determine the amount of time a cell will take to migrate through the pores of a permeable support. Some of these include chemoattractant type/concentration, biology of the cell, and barrier thickness/concentration. Therefore, it is critical to optimize the migration time for the specific conditions. For some applications, migration times of 2 hours are sufficient to obtain desired results while with other applications, 24 or 48 hours may be necessary. It is also important to keep in mind that longer migration incubation times often lead to higher spontaneous migration which can affect experimental results (Figure 4).

## Barrier Type and Concentration

Invasion assays require migration through some kind of biological barrier. Common barriers employed for invasion assays include extracellular matrix (such as BME or Corning Matrigel® matrix), collagen, fibronectin or laminin coatings as well as more defined complex extracellular or basement membrane extracts. The barrier chosen will depend on the model being examined as well as the concentration of the coating desired. To determine the appropriate coating concentration, try various coating concentrations in addition to some noncoated wells as controls. If the barrier is functioning properly there should be a statistical difference between the number of cells that migrate through the uncoated wells as compared to the cells that invade where there is a barrier (Figure 5).

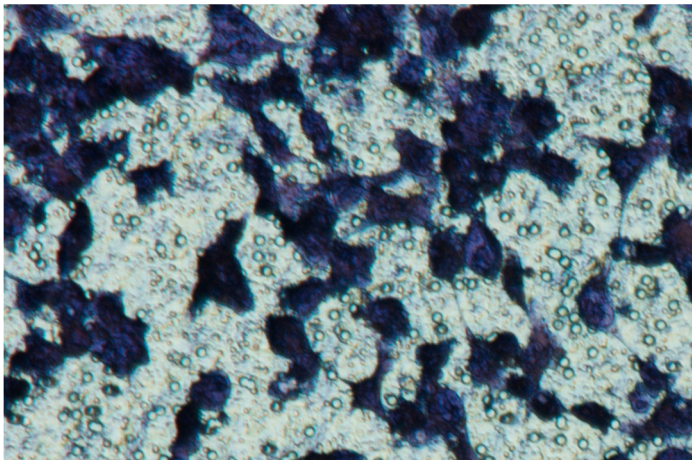


**Figure 1. Stained Migrating Cells.** Crystal violet stained HT1080's on 5.0 µm polycarbonate membrane (48X).

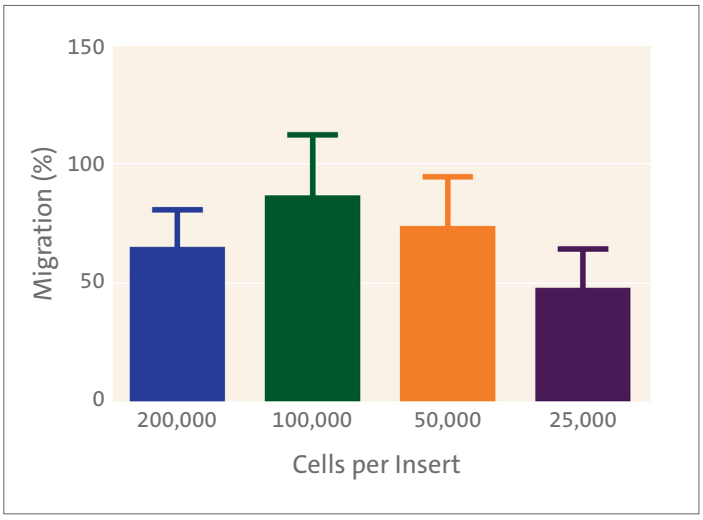



**Figure 2. Importance of Seeding Concentration in Migration Assays.** Optimizing HT1080 seeding concentrations for a 24-hour migration using 8.0 µm polycarbonate membrane.

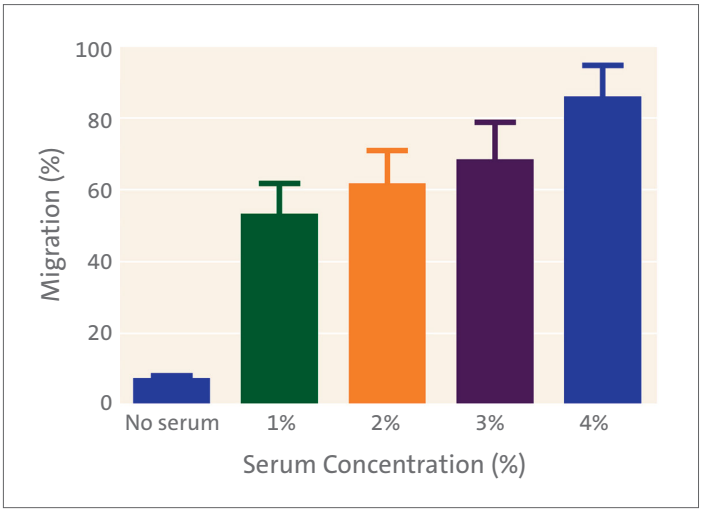



**Figure 3. Importance of Chemoattractant Concentration.** Optimizing serum concentrations for a 24-hour migration using HT1080's through 8.0 µm polycarbonate membrane.

## Preparing the Cells for Chemotaxis

In some applications it may be necessary to serum starve the cells for 24 to 48 hours prior to performing the migration assay. Serum starvation can increase the sensitivity of the cells to the chemoattractant and thus increase the migratory response as well as reduce potential variability. This can be accomplished by removing the medium from the culture flask, rinsing with PBS or HBSS, and then replacing the volume with serum free or reduced serum medium. In addition to starving the cells it may be necessary to optimize the method of harvesting the cells. Depending on the cell type and chemoattractant being used, some harvesting methods that use proteases (e.g., Trypsin) can damage cells or their receptors thereby reducing or inhibiting cell migration (Figure 6).

## Results and Discussion

Optimization for any cell-based assay is essential to achieving robust, reproducible results. Due to the complexity of migration and invasion assays optimization can be even more important. The overall health of the cells, seeding conditions, chemoattractant concentrations, membrane pore size, and migration/invasion incubation time are just a few critical factors for migration/invasion assays that need to be considered. Optimization of these critical factors not only will generate more accurate results but will also save time and money in the long run.

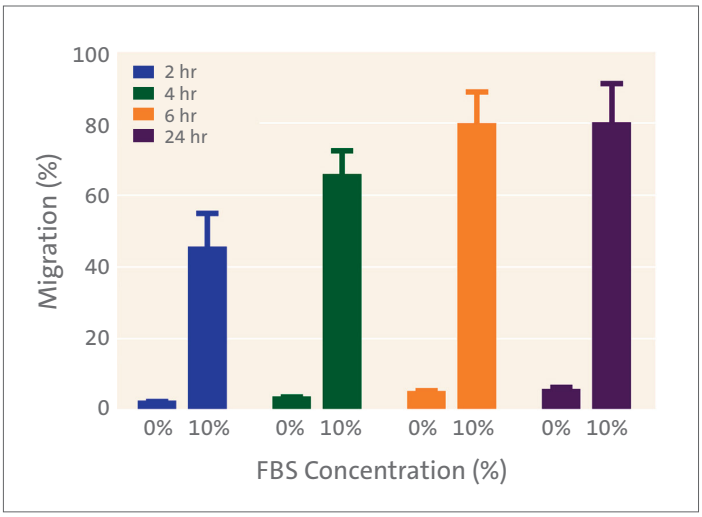



**Figure 4. Migration/Invasion Time.** Optimizing migration times for HT1080's migration through 8.0 µm polycarbonate membrane.

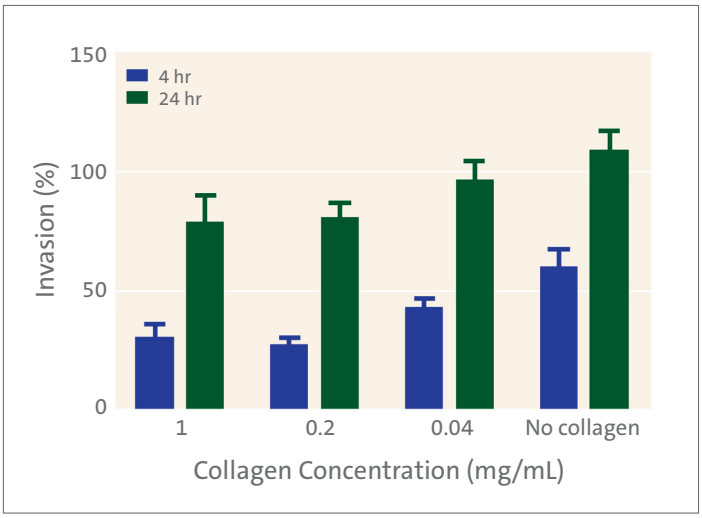



**Figure 5. Importance of Coating Concentration.** HT1080 invasion through a range of collagen concentrations on 8.0 µm polycarbonate membrane at either 4- or 24-hour migration time points.

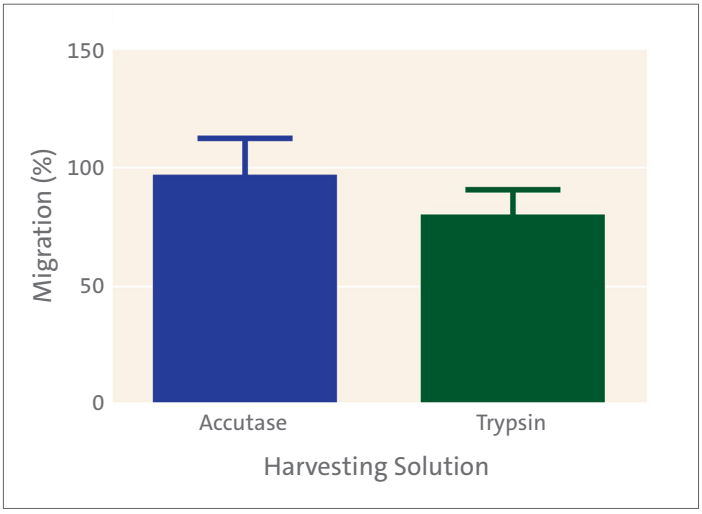



**Figure 6. Importance of Harvesting Solution.** 24-hour migration with HT1080 cells harvested with Accutase® or 0.05% Trypsin.

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