Cell Migration and Invasion Assay

Application Note

Application Introduction:

Vivogel ™ Matrix is a solubilized basement membrane extracted from the Engelbreth-Holm-Swarm (EHS) mouse tumor that comprises extracellular matrix proteins including laminin (glycoprotein), collagen IV, nidogen (glycoprotein), perlecan (heparan sulfate proteoglycan), and many other essential growth factors. **Vivogel ™ Matrix** has empowered applications such as stem cell culture, angiogenesis assays, and tissue engineering.

Cell migration and invasion assays are key techniques in cell biology, particularly in the study of wound healing, inflammation, and cancer metastasis. **Cell migration assay** is an analysis method of cell movement in response to a particular chemical stimulus. It involves determining if cells can move through a porous membrane. The most widely accepted cell migration technique is the Boyden Chamber assay. This assay uses a hollow plastic chamber, sealed at one end with a porous membrane. Cells are placed inside the chamber and allowed to migrate through the pores, to the other side of the membrane. Migratory cells are then stained and counted. **Cell invasion assay** is a more advanced analysis method of cell movement, which occurs through a filter with the molecules of the extracellular matrix (ECM). It determines if cells can move through both the membrane and an extracellular matrix (ECM). To analyze cell invasion, the transwell insert membrane is coated with Matrigel while in cell migration assays it is not. These assays provide valuable insights into how cells move and interact with their environment, and they're particularly useful in cancer research for studying how cancer cells invade surrounding tissues and spread to other parts of the body.

In this article, we use VivoMatter® CytoDrift ™ Permeable Cell Culture Inserts and Vivogel® Matrix to demonstrate how migration and invasion assays are conducted. You may also use Vivogel® Matrix GFR if you want to study cell invasion in a more controlled environment where you can add specific growth factors yourself, or if you want to reduce the influence of these factors on your results.

Procedure guidelines:

A.a. GENERAL HANDLING OF **Vivogel ™ Matrix**

Aliquots of **Vivogel ™ Matrix** are thawed as needed from -20/-80 ℃. All steps involving **Vivogel ™ Matrix** are to be finished on ice and require uses of pre-chilled tips and tubes. Freeze thaws should be minimized by aliquoting into one time use aliquots. It is extremely important that **Vivogel ™ Matrix** and all cultureware or media coming in contact with **Vivogel ™ Matrix** should be pre-chilled/ice-cold since **Vivogel ™ Matrix** will start to gel above 10 ℃

A.b. KEY MATERIALS AND REAGENTS

PRODUCT NAME	SUPPLIER	CATALOG #
Vivogel ™ Matrix	**Vivomatter Biotech**	VM001-10, VM001-PRF-10, VM002-10, VM002-PRF-10
MDA-MB-231 cells	ATCC	HTB-26
CytoDrift ™ Permeable Cell Culture Inserts, for 24-well plates with 8-μm pores	**Vivomatter Biotech**	VM101-24-8
Crystal violet solution 0.2%		
Fixation solution (4% paraformaldehyde)		

B. Procedures

1. **Seed cells and allow them to grow.** Plate MDA-MB-231 cells in an appropriate culture vessel using complete DMEM medium. Use seeding densities between 5×10^3 cells/cm^2 and 2×10^4 cells/cm^2. Replace culture medium every 2–3 days. Allow the cells to reach 70 – 90% confluency.

2. **(For invasion assay only) Preparation of Vivogel for coating.** One day before experiment, remove Vivogel from the freezer and place it in a refrigerator on ice. Thawing process will be completed after overnight-incubation at 4 °C. Dilute 1 part of Vivogel with 10 parts of cold PBS and thoroughly pipetting the mixture.

3. **(For invasion assay only) Coat CytoDrift ™ Permeable Cell Culture Inserts with Vivogel.** Place a tube of diluted Vivogel on ice. Invert the tube for a few times. Load 150 µL of diluted Vivogel per insert. Incubate the plate in a humidified incubator (37 °C, 5% CO_2) for 30 min - 1 hour to allow complete gelation. In some cases, you may observe small volumes of liquid remaining after the matrix solidifies. If desired, carefully aspirate excess liquid from inserts.

4. **Plating of cells on cell culture inserts.** Determine the cell count and dilute the cell suspension to the necessary seeding density in serum-free medium. Here, for the 24-well format, 100 µL of cell suspension containing 2 x 10^5 cells has been used. Add 650 µL of chemoattractant-containing medium to the bottom well. In this work DMEM with 10% FBS is used.

5. **Incubation.** Incubate the 24-well plate in a humidified incubator (37 °C, 5% CO_2) for 12 hours.

6. **Fixation**. Aspirate the medium from bottom wells and cell culture inserts. Wash both the inserts and bottom wells with PBS. Gently swab the inside of each insert using cotton swabs to remove cells that have not migrated/invaded. Be thorough around the edges of the membrane. Add 200 µL of fixation solution to the inserts and 500 µL to the wells, and incubate for 15 min at RT. Decant the fixation solution and rinse the inserts and well thoroughly.

7. **Staining**. Load 500 µL crystal violet solution into each bottom well and place the inserts into the wells. Watch out for any bubble beneath the inserts. After 15 minutes, decant the staining solution and rinse the insert with PBS thoroughly. And the inserts are ready for imaging.

Representative results:

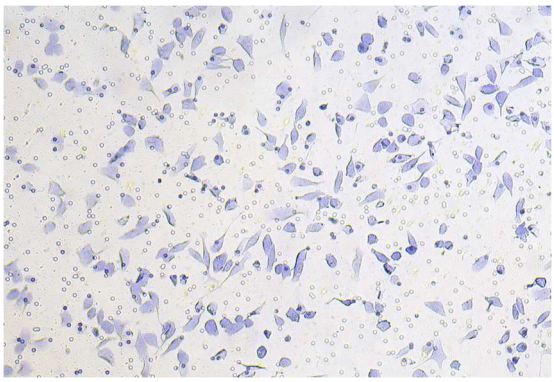

Figure 1. Migrated cells under microscope.