TREVIGEN®

amsbio

Management of Disease Through DNA Repair

Cell Invasion Assay Troubleshooting

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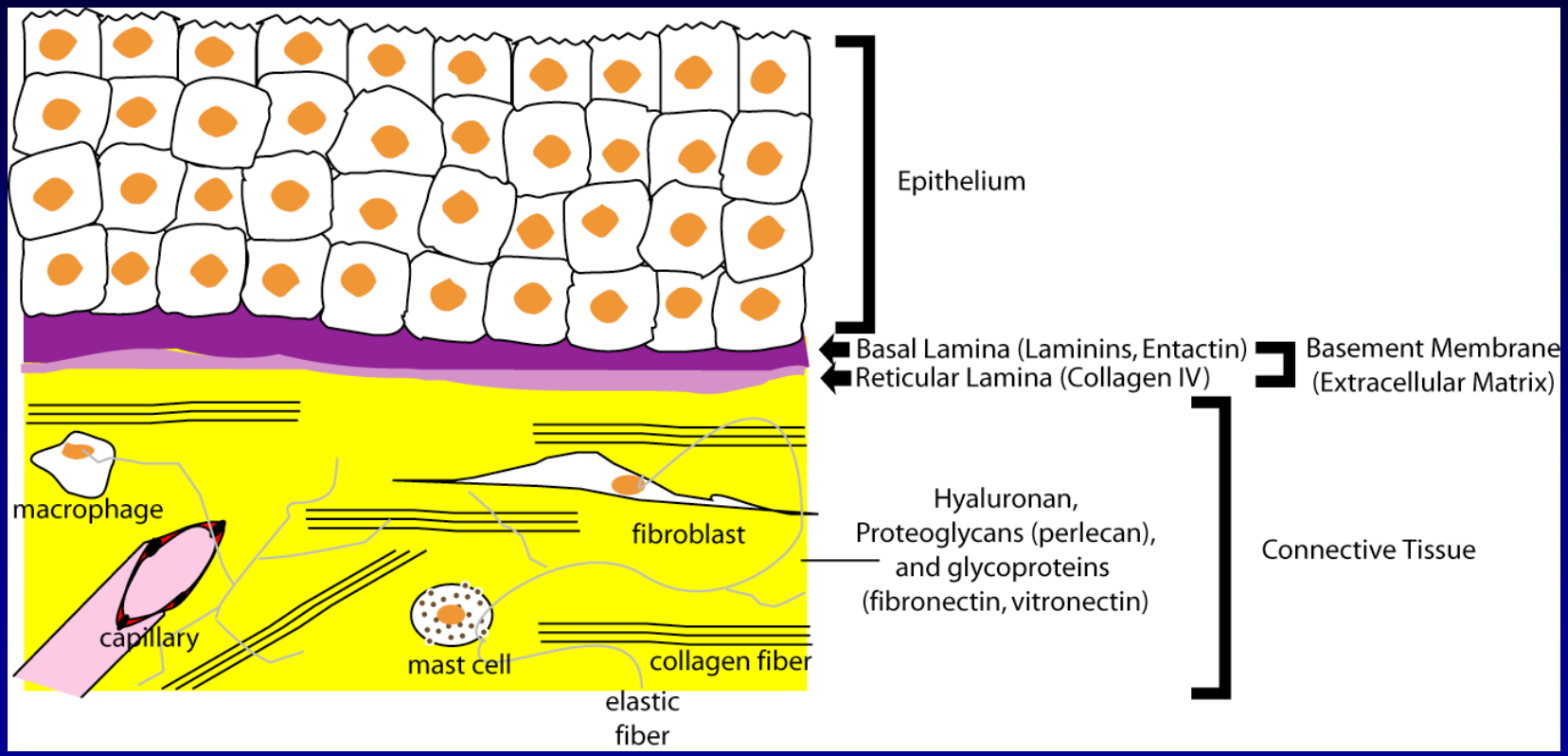
Cell Invasion Assay based on the ability of invasive and metastatic cancer cells, as well as endothelial cells to cross basement membranes and/or connective tissues in spread or to form new blood vessels.

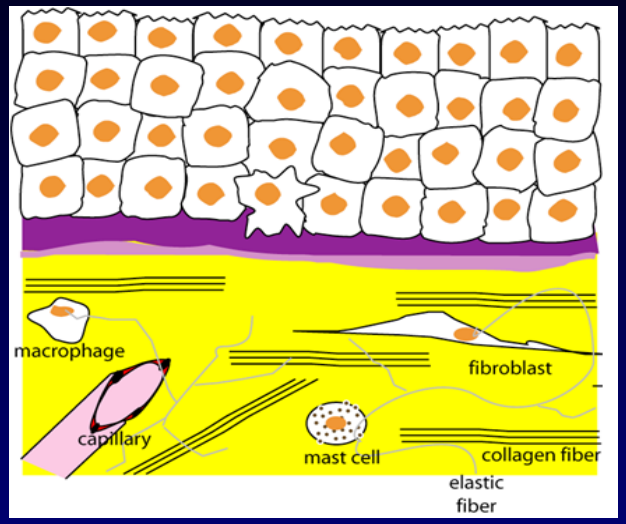
Physiological significance of cell invasion

- **Cancer**
  - **Initiation**
  - **Tumor Progression**
  - **Metastasis**
  - **Endothelial Cell Invasion**
- **Tissue Remodeling**
  - **Tissue Regeneration**
  - **Wound Healing**
  - **Endothelial Cell Invasion**

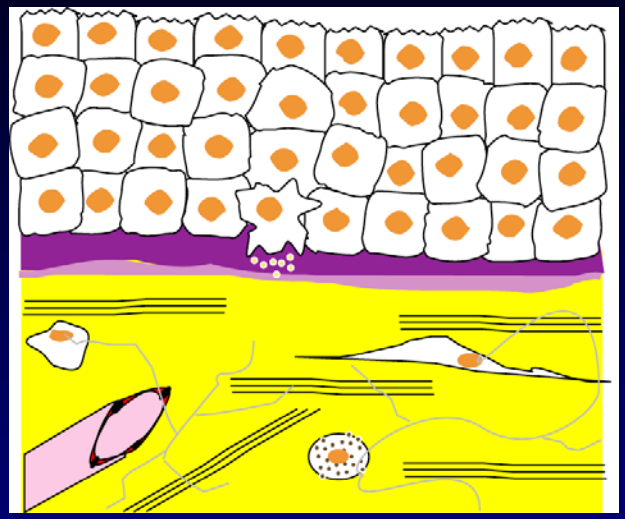
Normal Epithelium



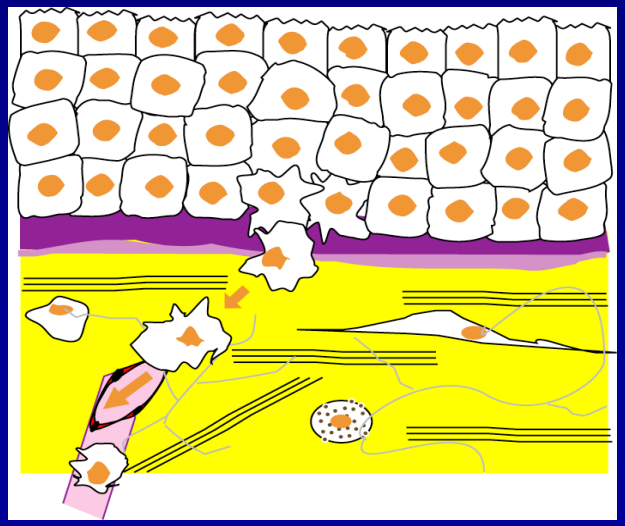
Epithelial to Mesenchymal Transition



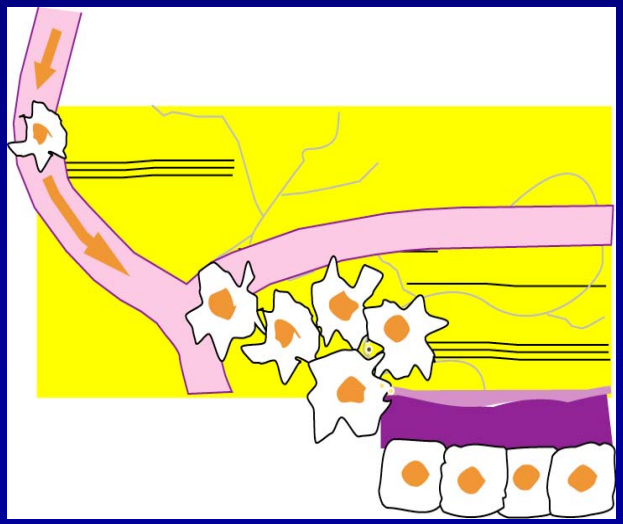
Matrix Metalloproteases Secretion



Proliferation Invasion and Intravasation



Extravasation and New Tumor Growth



- **Matrices and substrates for evaluating invasive potential *in vitro*.**
  - **Basement Membrane Extract (BME) (Reduced Growth Factor)**
  - **Laminin I**
  - **Collagen I**
  - **Collagen IV**

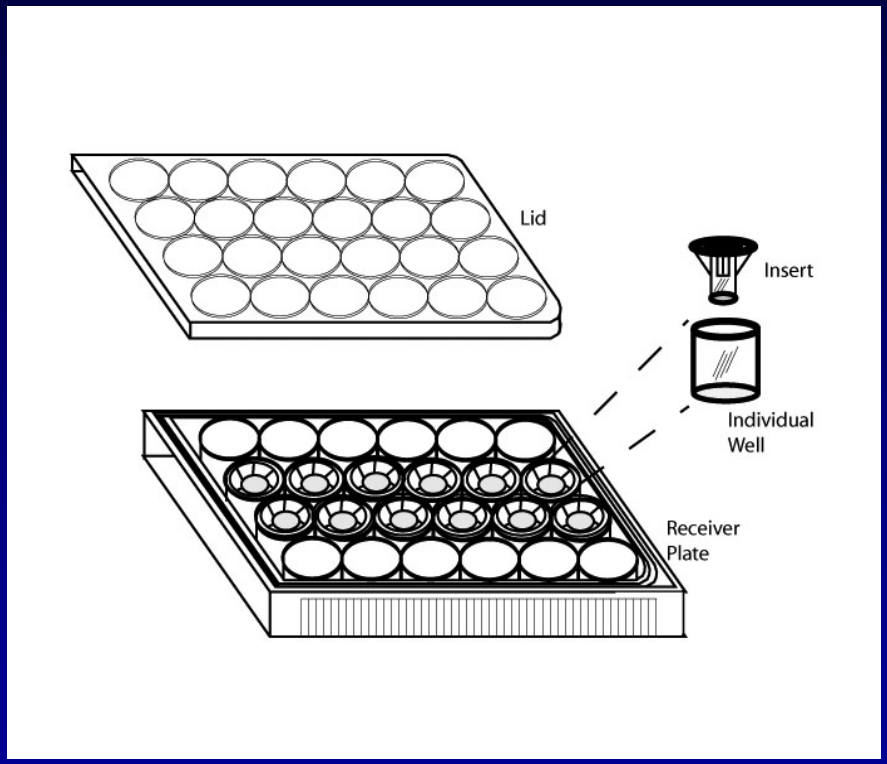
- **Cell Invasion Assay employs a simplified Boyden chamber-like design with an 8 micron polyethylene terephthalate (PET) membrane.**

- **Cell Invasion Assay is flexible, easy, quantitative, rapid and reliable way to quantify the invasive capacity of many cell types.**

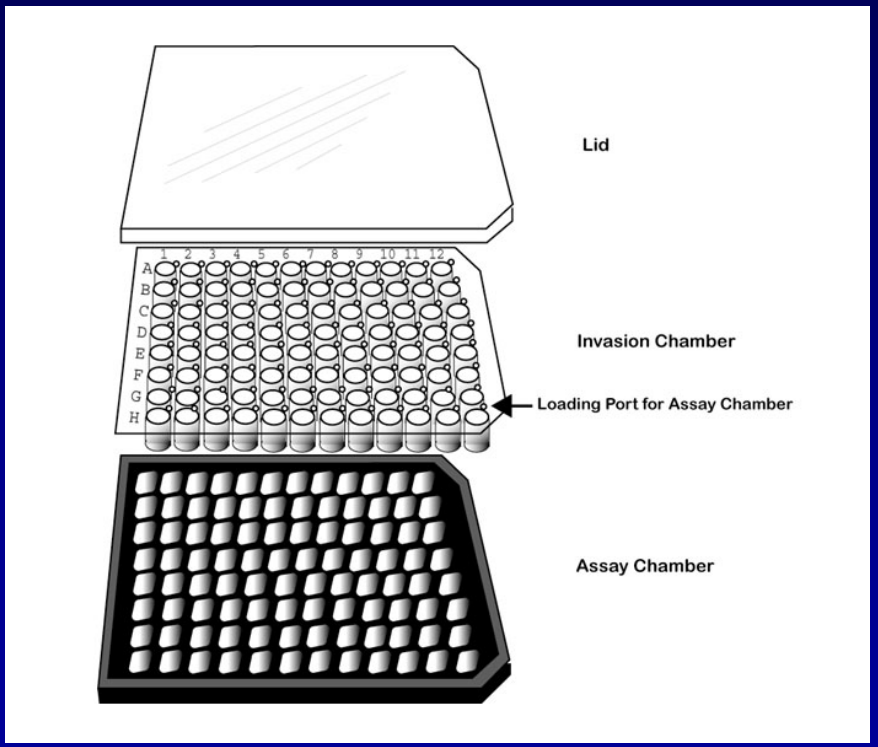
- **Cell Invasion Assay is used to measure tumor cell invasion and can be successfully used to study endothelial activation and angiogenesis.**

Cell Invasion Assay Chambers

24-well plate



96-well plate



Assay originally described by Albini et al, Cancer Research, 1987 for tumor cells but now also adapted for endothelial cells

Cell Invasion Assay (in 96-well format)

Add 50 µl of coating protein solution per well to the top chamber

→

Incubate cell invasion chamber overnight at 37°C in a cell culture incubator

→

Add 150 µl of medium ± chemoattractants and/or inhibitors to the bottom chamber wells

↓

Incubate cell invasion chamber for 6-24 hours in a cell culture incubator

←

Wash wells, add 150 µl per well of Cell Dissociation solution with 2 µM Calcein AM into bottom chamber, and incubate invasion chamber 1 hour at 37°C

←

Read fluorescence using Plate Reader

↑

Add 100 µl of diluted cells per well to the top chamber

←

Dilute cells in serum-free medium ± inhibitors

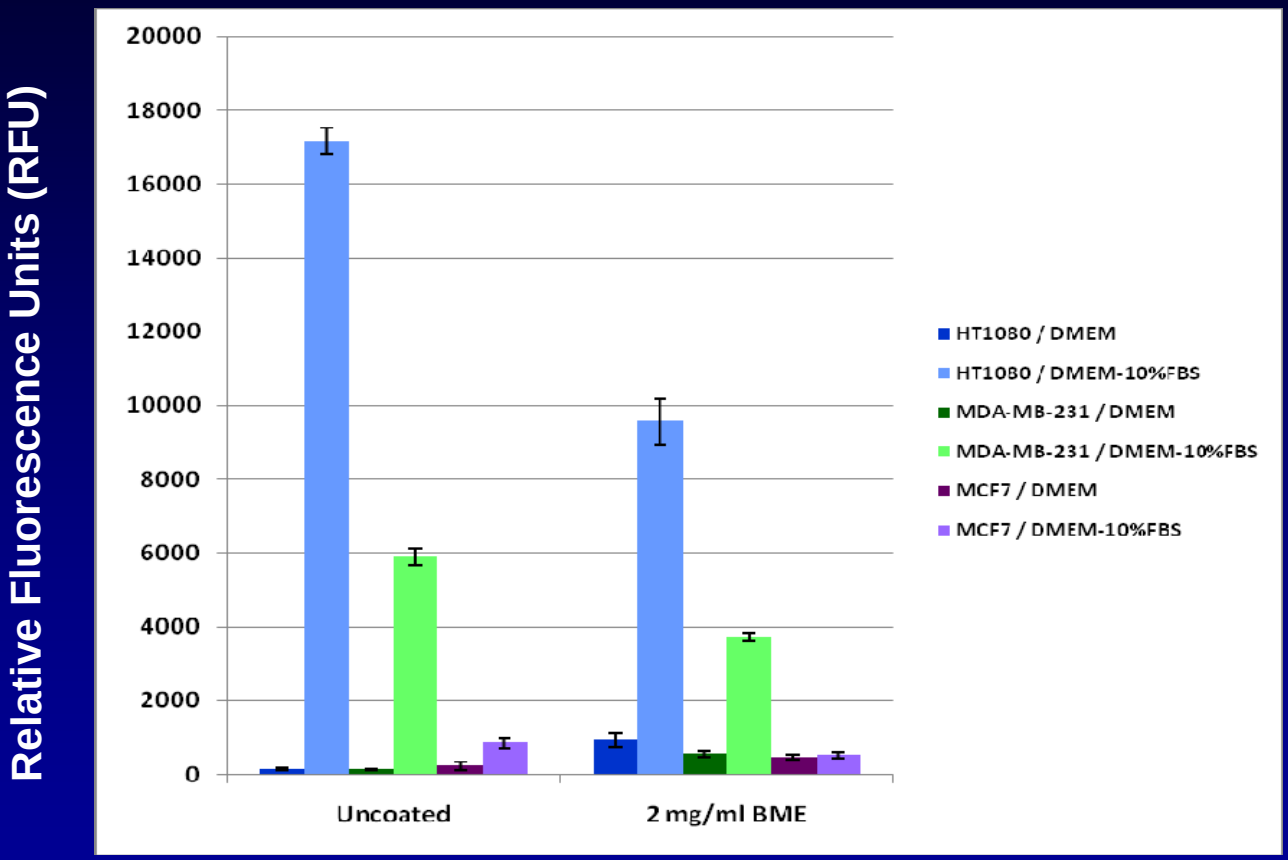
←

Trypsinize, harvest and count cells

Variables Affecting Cell Invasion Assay

- **Type of the cells (tumor, cancer metastatic, endothelial, etc.)**
- **Extracellular matrix environment:**
  - **Type of the extracellular matrix proteins (Collagen IV, Laminin I, BME or Collagen I)**
  - **Thickness/density of the extracellular matrix barrier**
- **Type of the chemoattractants or angiogenic factors**
- **Length of the assay (hours)**
- **Cell seeding density (cells/well)**

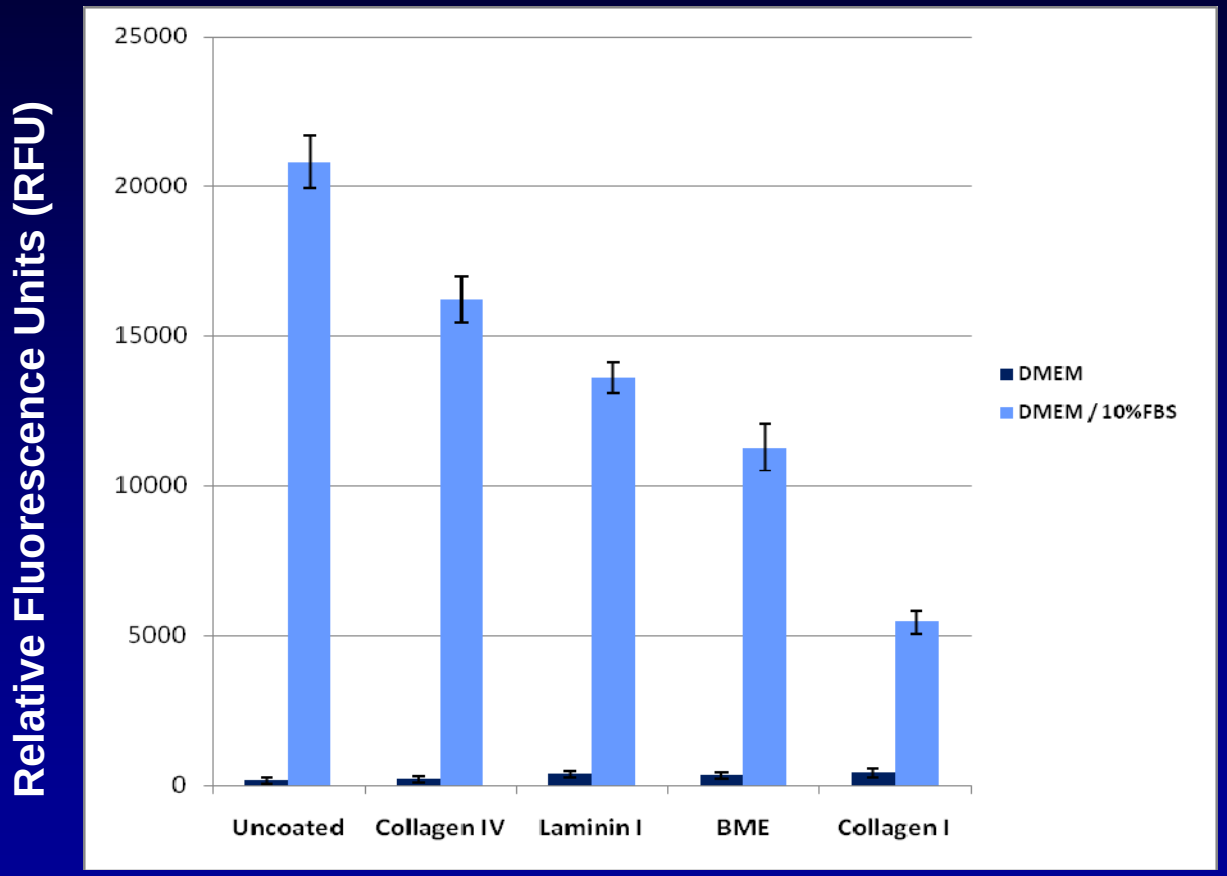
Various cell lines have different invasive potential which correlates with their malignant potential



Quantification of the ability of HT1080 cells, MDA-MB-231 cells and MCF7 cells to cross a barrier 8 micron polyester filter uncoated or coated with 2 mg/ml Basement Membrane Extract (BME) in response to FBS. Cells were plated at 20,000 cells/well in a 96-well cell invasion chamber and incubated for 24 hours.

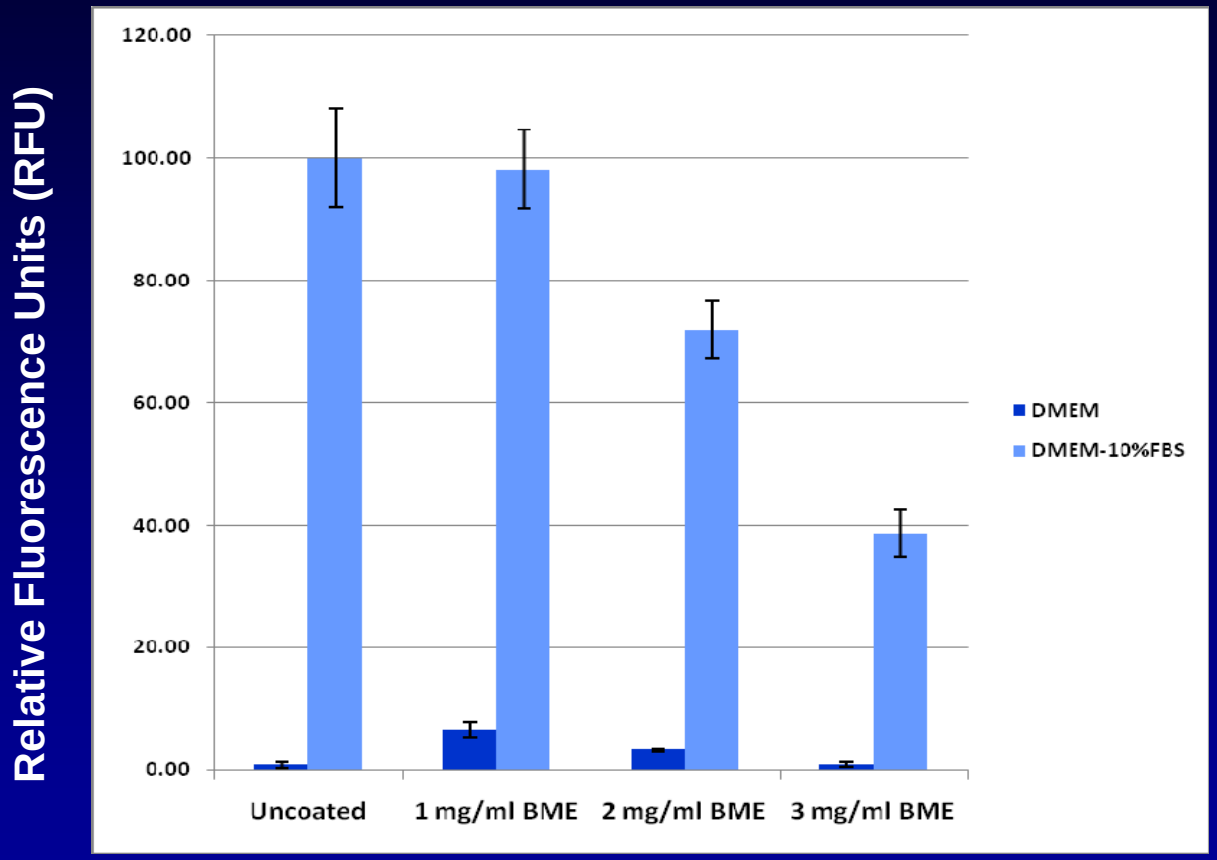
NOTE: Cells require a chemoattractant to migrate and to invade.

Cells have different ability to invade through various type of barrier



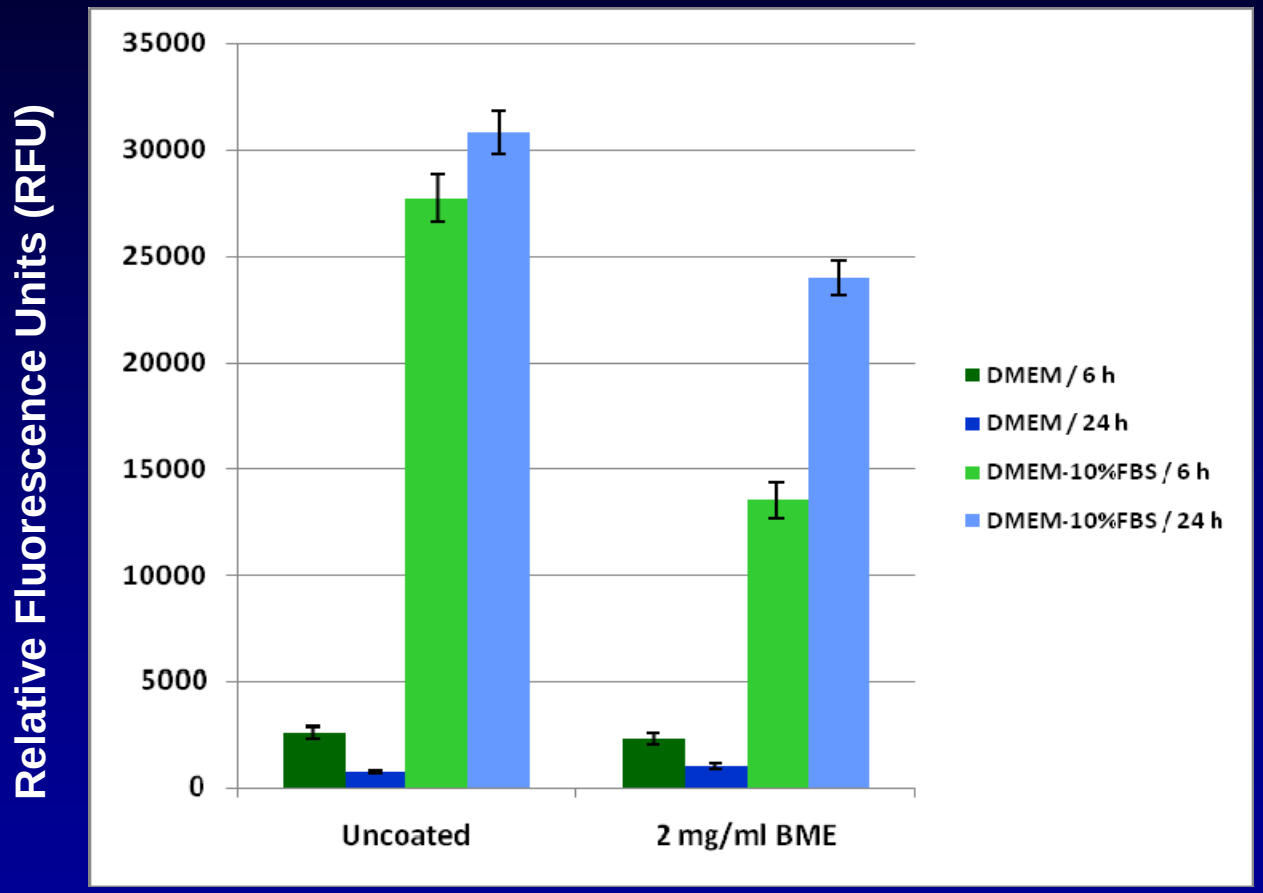
Quantification of the ability of HT1080 cells to cross a barrier 8 micron polyester filter uncoated or coated with various extracellular matrix proteins in response to Fetal Bovine Serum (FBS). Cells were plated at 20,000 cells/well of 96-well cell invasion chamber and incubated for 24 hours.

Level of invasion depends on the thickness of the barrier (protein concentration of the coating solution)



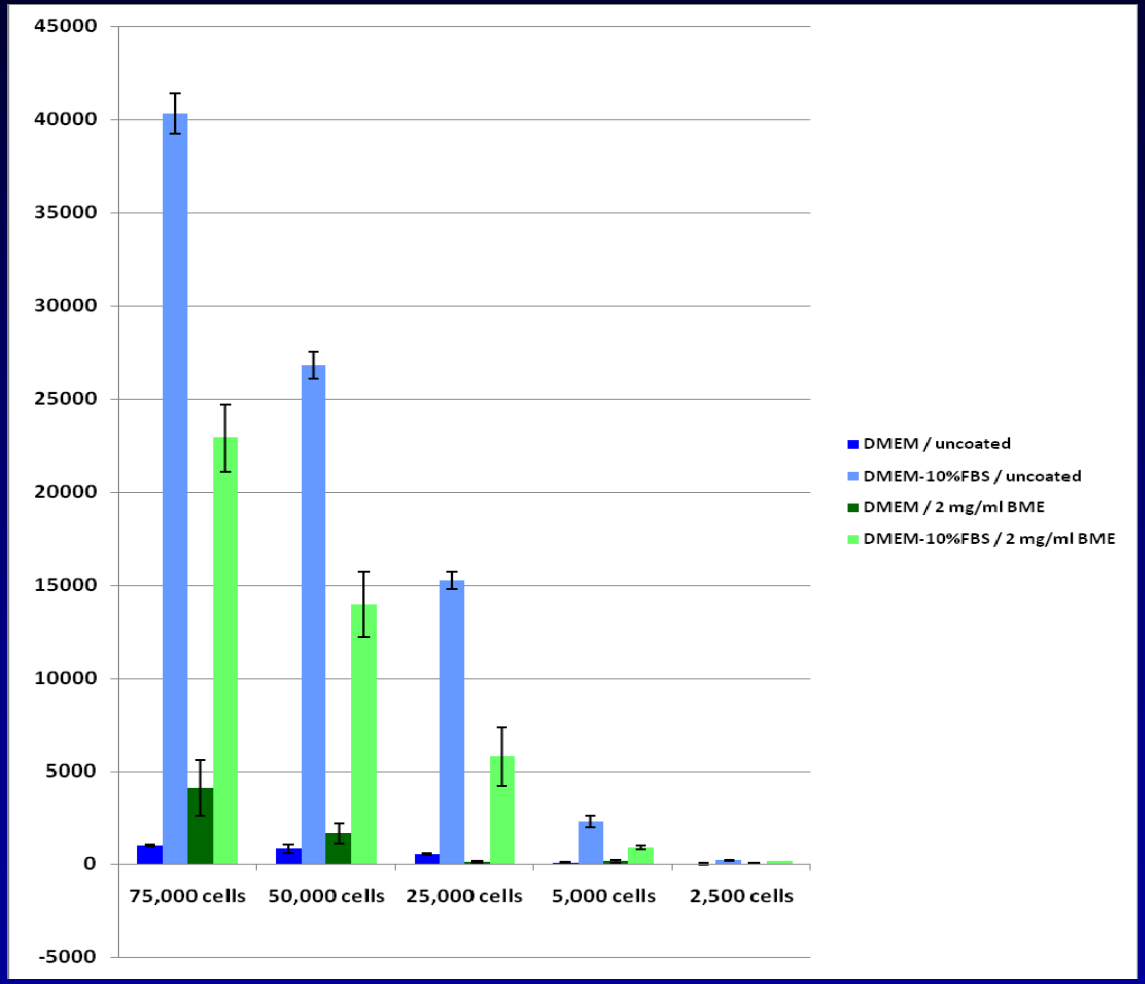
Relative quantification (as a percent of the control) of HT1080 cells to cross a barrier 8 micron polyester filter uncoated or coated with BME solution at various concentration in response to FBS. Cells were plated at 20,000 cells/well. Cell invasion chamber was incubated for 24 hours in the cell culture incubator. Note the more BME, the less invasion.

Invasion level is time-dependent



Quantification of HT1080 cells (40,000 cells/well of 96-well) to cross an 8 micron polyester filter, uncoated or coated with 2 mg/ml BME solution in response to FBS during 6 hours or 24 hours period.

Cell density affects the invasion assay



HT1080 cells were seeded at the various concentration onto 8 micron polyester filter, uncoated or coated with 2 mg/ml BME solution. 96-well cell invasion chamber was incubated for 24 hours in the cell culture incubator. NOTE Recommended cell number is 20,000 - 40,000 cells.

Summary

The success of your invasion assay involves attention to variables:

- **Chemoattractant used**
- **Cell type**
- **Type of barrier**
- **Thickness of the barrier**
- **Cell density**
- **Time of the assay**

Each assay should be customized by the investigator based on these variables. The conditions for many tumor cells have generally already been published.

Ordering Information:

Cultrex 24 Well BME Cell Invasion Assay	*3455-024-K*
Cultrex 24 Well Collagen I Cell Invasion Assay	*3457-024-K*
Cultrex 24 Well Collagen IV Cell Invasion Assay	*3458-024-K*
Cultrex 24 Well Laminin I Cell Invasion Assay	*3456-024-K*
Cultrex 96 Well BME Cell Invasion Assay	*3455-096-K*
Cultrex 96 Well Collagen I Cell Invasion Assay	*3457-096-K*
Cultrex 96 Well Collagen IV Cell Invasion Assay	*3458-096-K*
Cultrex 96 Well Laminin I Cell Invasion Assay	*3456-096-K*
Cultrex Endothelial Cell Invasion Assay	*3471-096-K*

Precoated Invasion Assay Kit

CultreCoat® 24 Well BME Cell Invasion Assay	*3460-024-K*

Cell Migration:

Cultrex 24 Well Cell Migration Assay	*3465-024-K*
Cultrex 96 Well Cell Migration Assay	*3465-096-K*