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

Extravasation and New Tumor Growth

Proliferation Invasion and Intravasation
• Matrices and substrates for evaluating invasive potential \textit{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 \textbf{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

Read fluorescence using Plate Reader

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

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

Trypsinize, harvest and count cells

Dilute cells in serum-free medium ± inhibitors

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

<table>
<thead>
<tr>
<th>Assay Description</th>
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<tr>
<td>Cultrex 24 Well BME Cell Invasion Assay</td>
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<td>Cultrex 24 Well Collagen I Cell Invasion Assay</td>
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<td>Cultrex 24 Well Collagen IV Cell Invasion Assay</td>
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<td>Cultrex 24 Well Laminin I Cell Invasion Assay</td>
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<td>Cultrex Endothelial Cell Invasion Assay</td>
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**Precoated Invasion Assay Kit**

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**Cell Migration:**

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