Assay Methods: Cell Invasion Assay

Protocol

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The Invasion Assay provides an *in vitro* system to study cell invasion of malignant and normal cells. Specific applications include assessment of the metastatic potential of tumor cells,[1] inhibition of metastasis by extracellular matrix components[2] or antineoplastic drugs (TAXOL®)[3], altered expression of cell surface proteins[4], or matrix metalloproteinases[5] in metastatic cells; and invasion of normal cells such as embryonic stem cells[6], cytotrophoblasts[7], endothelial cells[8], and fibroblasts[9]. Invasion studies have been successfully performed on a variety of tumor cells (cell lines and primary tumors) including melanomas, glioblastomas, astrocytomas, osteosarcomas, fibrosarcomas, and adenocarcinomas of the lung, prostate, breast, ovary, and kidney.

Invasion chambers coated with Corning® Matrigel® matrix provide cells with the conditions that allow assessment of their invasive capacity *in vitro*. Corning Matrigel matrix serves as a reconstituted basement membrane *in vitro*, occluding the pores of the membrane and blocking non-invasive cells from migrating through the membrane. In contrast, invasive cells (malignant and non-malignant) secrete proteases that enzymatically degrade the Matrigel matrix and enable invasion through the membrane pores. The membrane may be processed for light and electron microscopy and can be easily removed after staining. The following procedure has been optimized using HT-1080 human fibrosarcoma cells on Matrigel matrix-coated inserts (8.0 µm pore diameter). Results may vary depending upon the experimental conditions including the cells[10], medium, incubation time, cell seeding density, and chemoattractant. Therefore, the conditions including cell culture insert pore size[10] should be optimized for each system.

Materials

- Corning BioCoat® cell culture inserts packaged in companion plates (24-well, Corning 354578), or individual inserts (24-well, Corning 353097). Falcon® cell culture inserts are also available in various pore sizes.
- Falcon cell culture insert companion plates (24-well, Corning 353504)
- Corning Matrigel basement membrane matrix (10 mL vial; standard Corning Matrigel matrix (Corning 354234), or Growth Factor Reduced Corning Matrigel matrix (Corning 354230)
- Chemoattractant such as 5% FBS (fetal bovine serum) in tissue culture (TC) medium (e.g., Corning 35-011-CV)
- Bicarbonate-based culture medium such as DMEM (serum-free) (Corning, various formulations)
- Tris; Sodium Chloride (coating buffer)
- 0.2 µm filter unit (e.g., Corning 431222)
- Diff-Quik staining kit (Siemens B4132-1A), or other suitable fixative and stain
- Microscope with (optional) camera
- Cotton swabs
- Sterile forceps
- Positive displacement pipet
- Syringes

Procedure

1.0 Coating Protocol

NOTE: The following steps must be performed using aseptic technique. Corning® Matrigel® matrix should be reconstituted, aliquoted, and stored as recommended in the product specification sheet. The concentration of Matrigel matrix is lot-specific and found on the Certificate of Analysis. Matrigel matrix and the coating buffer must be kept ice-cold during the entire procedure.

1.1 Prepare coating buffer: 0.01M Tris (pH 8.0), 0.7% NaCl. Filter using a 0.2 µm sterile filter unit.

1.2 Thaw Matrigel matrix aliquot on ice at 4°C. Once thawed, swirl vial to ensure that material is evenly dispersed. Keep product on ice and handle using sterile technique.

NOTE: Allow the coating buffer to cool for two hours in an ice bath in a cold room, or refrigerator. Any pipets, syringes, or containers that will come in contact with Matrigel matrix must be chilled prior to use. Syringes and pipets can safely be placed on ice if placed within a plastic bag.

1.3 Prepare coating solution: Mix Matrigel matrix (final concentration of 200 to 300 µg/mL) with coating buffer in a final volume of 2.0 mL. Thoroughly mix the coating solution containing Matrigel matrix by gently swirling, and then place the tube on ice.

NOTE: This quantity of Matrigel matrix in the coating solution should be sufficient to coat twelve 24-well individual inserts. Scale accordingly for the number of inserts needed for the experiment. For each new syringe, fill halfway with Matrigel matrix, expel, and then fill completely to coat. If using a positive displacement pipet, a larger volume of Matrigel matrix will be needed to prevent or minimize bubble formation.

Insert Coating (Invasion Chambers)

1.4 Under the hood, remove the lid from a 24-well insert plate (Corning 354578) or unwrap individual inserts (Corning 353097), and use sterile forceps to transfer the required number of inserts into the wells of a Falcon® TC-treated companion plate (Corning 353504).

1.5 Use a sterile syringe or pipet to carefully add 0.1 mL of the diluted Matrigel matrix coating solution to each insert. Minimize contact of the Matrigel matrix with the side walls of the insert. Repeat coating process with the remaining inserts.

1.6 Incubate plates with the coated inserts (invasion chambers) at 37°C for 2 hours. Just before use, carefully remove the remaining liquid (coating buffer) from the insert membrane without disturbing the layer of Matrigel matrix on the membrane. The coated invasion chambers are now ready for use. Do not let the Matrigel matrix layer dry out.

2.0 Invasion Protocol

2.1 Prepare an equal number of control (uncoated) inserts by using sterile forceps to transfer the inserts into empty wells of a Falcon TC-treated companion plate.

2.2 To prepare cells for the invasion assay, culture the cells according to your requirements (e.g., media, serum concentration, confluency). For HT-1080, we recommend culturing to approx. 70-80% confluency prior to sub-culturing into the invasion chamber.

2.3 Prepare cell suspensions in culture medium containing 5×10^4 cells/mL for the 24-well invasion chambers.

NOTE: To determine the optimal seeding density for your cell type on a porous membrane growth surface, use a range (cells/cm^2) that brackets the typical seeding density used on nonporous surfaces (i.e., flasks, dishes, and plates). For example, if you currently seed at 1×10^5 cells/cm^2, seed at 5×10^4 to 5×10^5 cells/cm^2 to determine the optimal seeding density.

2.4 Add 0.5 mL of cell suspension (2.5×10^4 cells) to each 24-well invasion chamber.

2.5 Add chemoattractant (0.75 mL) to the wells of the Falcon TC-treated companion plate via the access port.

NOTE: Be sure that no air bubbles are trapped beneath the insert membranes.

2.6 Incubate cell invasion chambers overnight in a humidified tissue culture incubator at 37°C, 5% CO_2 atmosphere.

3.0 Measurement of Cell Invasion

Removal of Non-Invading Cells

3.1 Insert a cotton swab moistened with medium into the top of the Matrigel matrix-coated insert (apical side), and apply gentle but firm pressure while gently rubbing the area. Repeat with a second swab moistened with medium.

Staining of Cells

NOTE: The cells on the lower surface of the membrane are stained with Diff-Quik™ stain. The Diff-Quik kit contains a fixative and two stain solutions. Staining is accomplished by sequentially transferring the inserts through the three Diff-Quik solutions and two water rinses. The cell nuclei stain purple and the cytoplasm stains pink.

3.2 Add each solution (0.5 mL) from the Diff-Quik kit to three rows of a Falcon TC-treated companion plate. Add distilled water (0.5 mL) to two 24-well plates, or (150 mL) to two beakers.

3.3 Sequentially transfer the inserts through each stain solution and the two plates (or beakers) of water. Allow approximately 2 minutes in each solution.

3.4 Allow the insert membrane to air dry.

NOTE: Alternatively, cells may be fixed and stained with 100% methanol and 1% Toluidine Blue, respectively (see below). Alternative staining procedures include fixation followed by hematoxylin and eosin staining or crystal violet.

Add 0.5 mL of 100% methanol to the appropriate number of wells of a Falcon TC-treated companion plate. In a separate plate, add 0.5 mL of 1% Toluidine Blue in 1% borax to the appropriate number of wells. Add distilled water (0.5 mL) to two 24-well plates or (150 mL) to two beakers. Transfer inserts into the methanol for 2 minutes, then into the Toluidine stain for 2 minutes. Rinse insert membrane in the two 24-well plates or beakers of distilled water to remove excess stain. Allow the insert membranes to air dry.

Counting of Invaded Cells

NOTE: Cell counting can be facilitated by image capture of cells attached to the basal surface through a microscope. Direct counting of the cells is also acceptable.

3.5 Observe and/or image the invaded cells under the microscope at approximately 40X to 100X total magnification depending on cell density. Count cells in several fields in triplicate.

NOTE: When counting cells, choose fields in the center of the insert membrane as well as fields at the periphery of the membrane for an accurate representation of the cell number throughout the membrane. Data is expressed as the percent invasion through the Corning® Matrigel® matrix and membrane relative to the migration of cells through the uncoated membrane.

3.6 Determine the Percent Invasion:

$$\% \text{ Invasion} = \frac{\text{mean number of cells invading through Corning Matrigel matrix-coated insert membrane}}{\text{mean number of cells migrating through uncoated insert membrane}} \times 100$$

References

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