# Cell invasion assay

## 1、Experimental principle

Transwell chamber is in the culture plate, the chamber is called the upper chamber, and the culture plate placed in the chamber is called the lower chamber. The bottom of the upper chamber is a layer of polycarbonate membrane, which is permeable and separates the culture medium in the upper chamber from the culture medium in the lower chamber. The culture medium in the lower chamber can affect the cells in the upper chamber, so as to study the influence of the liquid in the lower chamber on the growth and migration of cells. The cells are inoculated into a hypotrophic medium, and the membrane pores are usually covered with Matrigengel matrix. To mimic the extracellular matrix, the tumor cells must secrete hydrolytic enzymes and move through the membrane with Matrigengel matrix. The invasion ability of tumor cells can be reflected by counting the number of cells entering the lower chamber.

With the application of different pore sizes and different treatments of polycarbonate membranes, various aspects of co-culture, cell chemotaxis, cell migration and cell invasion can be studied.

## 2、 Experimental instruments and materials

2.1 Instruments: Biosafety cabinet, carbon dioxide incubator, low temperature horizontal centrifuge, inverted microscope, low temperature refrigerator.

2.2 Materials: Pipette (10μL, 200μL, 1000μL), sterile pipette tips (10μL, 200μL, 1000μL), 1.5mL Sterile EP tube and Transwell chamber, pre-cooled 24-well plate

2.3 Reagents: Mogengel Matrigengel matrix, basic medium, complete medium containing 10% fetal bovine serum, Trypsin digestion solution, 1×PBS, 0.1% crystal violet dye solution, 4% paraformaldehyde

## 3、Experimental contents and methods

3.1 Preparation before experiment:

- Put the Matrigengel in the ice box and put it in the refrigerator at 4℃ so that the Matrigengel can slowly melt overnight(Do not allow this product to warm up above 4° C during manipulation. Keep the product on ice and dilute using ice-cold solutions or cell suspensions.);
- Prepare enough complete medium containing 10% fetal bovine serum;

- 1.5 mL Sterile EP tube, Transwell Chambers and basic medium were be pre-cooled at 4℃ in advance.

3.2 Plating with Matrigengel matrix (ice operation) :

3.2.1 Diluting Matrigengel matrix: Take 10μL Matrigengel matrix into precooled 1.5mL Sterile EP tube, then add 80μL precooled basic medium, and mix thoroughly with the pipette. (The ratio of Matrigengel matrix：base medium is 1:8)

3.2.2 Absorb 60μL diluted Matrigengel matrix, add it vertically into the upper chamber of Transwell, and evenly lay it on the bottom. The 60μL basic medium was extracted from another compartment in the upper compartment as a blank control. It was incubated in an carbon dioxide incubator to polymerize the Matrigengel matrix into films. (Note: Do not produce bubbles during the gel plating process)

3.3 Preparation of cell suspension

3.3.1 Cells can be starved of serum for 12-24 hours before preparing cell suspension to further remove the influence of serum. (This step is optional, if the cell migration ability is not strong, you can consider this step to increase the serum induction of cells.)

3.3.2 Cells with a confluent degree of 70%-80% were taken for digestion, and the waste liquid was centrifuged, then the cells were re-suspended with basic medium, and the cell density was adjusted to 50,000 per well after counting. (Cell density can be adjusted according to Transwell migration assay of different cell lines.)

3.4 Inoculated cells

3.4.1 500μL complete medium containing 10% fetal bovine serum was added into the lower chamber of 24-well plate. The Transwell chamber was then placed in the 24-well plate with tweezers. (Note: Bubbles often occur between the lower culture medium and the chamber. Once bubbles occur, the chemotaxis of the lower culture medium will be weakened or even disappear.)

3.4.2 Add 200μL cell suspension per well into the upper chamber of Transwell.

3.4.3 Fixed staining can be carried out after 24 hours in the incubator. (The time point can be determined according to the cell invasion ability, generally 24-48 hours, also need to consider the effects of cell state and digestion time on cells. In addition, it is recommended to take the culture

plate out of the incubator for observation 1-2 hours after the cells are inoculated to ensure that there are no big bubbles.)

3.5 Cell Fixation

3.5.1 Take out the Transwell chamber, discard the medium, and gently wipe the Matrigengel matrix and cells in the upper chamber with a cotton swab.

3.5.2 Add 600μL 4% paraformaldehyde 600μL into the clean hole of the 24-well plate, place the chamber and fix it for 20-30 minutes.

3.6 Cell staining and counting

3.6.1 Discard the fixative and wash the chamber gently in a 6cm dish containing PBS for one time. (Be careful not to touch the bottom of the chamber)

3.6.2 Dye with 0.1% crystal violet for 5-10 minutes, gently wash with PBS for 3 times, remove the crystal violet that is not bound to cells, gently wipe the upper side of the chamber with a cotton swab, and erase the dye that is not specifically bound to the upper surface of the chamber for subsequent microscopic examination.

3.6.3 After proper air drying, 5 fields were selected under the microscope to observe and count the cells.