Transwell assay protocol

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Ordering information

ITEMS	CATALOG #
ECM gel	Sigma-Aldrich, catalog number: E1270
8 μm pores transwell plate	Merck KGaA, catalog number: PI8P01250

Invasion Protocol:

1. Grow cells in DMEM supplemented with 10% FBS.

2. Thaw ECM gel overnight at 4 °C and keep on ice.

3. Chill Millicell insert and plate to 4 °C, keep on ice.

4. Dilute ECM gel in ice-cold, serum-free DMEM to a final concentration of 1mg/ml.

Note: ECM gel final concentration may vary, depending on the cell type studied.

5. Add 40 μl ECM gel from step 4 into the upper compartment of the insert. Immediately incubate the plate, with insert and ECM gel inside, at 37 °C for 2 h. This allows the liquid ECM gel to solidify.

6. Wash cells with 1x PBS and resuspend in serum-free DMEM. To the well of the plate (lower compartment), add 0.6ml DMEM supplemented with 10% FBS as attractant.

7. Position the insert into the well, with the bottom of the insert merged in medium.

8. Gently add 1.0-5.0x10^5 cells (no more than 0.2ml medium) from step 7 to the upper compartment of the insert. Incubate the plate at 37 °C for 16-48h.

9. After the incubation period, take the insert out carefully. Fix the cells on the lower side of the insert membrane with 5% glutaraldehyde for 10 min, followed by staining with 1% crystal violet in 2% ethanol for additional 20 min. Wash the insert in PBS for several seconds to remove excess dye,

10. Cells and the gel in the upper compartment of the insert need to be gently removed by gently wiping the upper side of the membrane with a cotton swab.

11. Dry the insert completely, count the number of cells on the lower side of the filter under a microscope.

12. Randomly choose different views and take average counting.

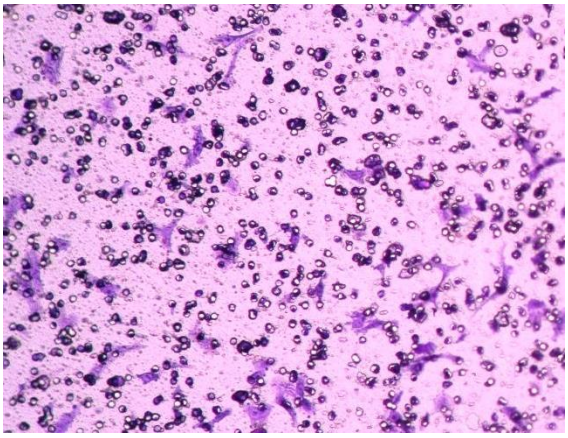
Migration Protocol:

1. Cells (5×10^4 -2 $x10^5$ cells per well) were seeded in top chambers of the transwell plates in FBS-free media with membrane inserts without matrigel coated. To the well of the plate (lower compartment), add 0.6ml DMEM supplemented with 10% FBS as attractant.

2. Incubate the plate at 37 °C for 16-48h.

3. The Cells that migrate to the lower surface of the membrane are fixed, stained with crystal violet and observed as the 9-12 steps in invasion protocol mentioned.

Example:



Wound healing protocol

1. Grow cells in DMEM supplemented with 10% FBS.

2. Seed cells into 6-well tissue culture plate at a density that after 24 h of growth. Do not change the medium.

3. Gently and slowly scratch the monolayer with a new 1 ml pipette tip across the center of the well. While scratching across the surface of the well, the long-axial of the tip should always be perpendicular to the bottom of the well.

Note: The resulting gap distance therefore equals to the outer diameter of the end of the tip. The gap distance can be adjusted by using different types of tips. Scratch a straight line in one direction. Scratch another straight line perpendicular to the first line to create a cross in each well.

4. After scratching, gently wash the well twice with PBS to remove the detached or dead cells. Replenish the well with fresh medium.

5. Grow cells for additional 48 h (or the time required if different cells are used). Set the same configurations of the microscope when taking pictures for different views. The gap distance or closure area can be quantitatively evaluated.