Transwell Cell Migration/Invasion Matrigel Assay

(Yuxi Su, 7-6-2008)

I. Reagents and Materials

1. Dilute matrigel (BD) at 0.1-1.0 mg/ml with serum free media (the media with which you use for your cells of interest)

Note: we usually stock matrigel at 1.0 mg/ml. **KEY IS TO PUT EVERYTHING IN FREEZER** before dilute original stock and only thaw once after that. BD Biosciences sells Matrigel at high concentration and Growth-factor reduced matrigel. Tips, pippettes, and containers to be aliquoted in should be at -20 overnight and thaw original vial on ice. As soon as thawed use serum-free media straight from refrigerator and dilute entire bottle in serum-free media at 1.0mg/ml than aliquot in eppendorf tubes and freeze immediately. **KEY IS TO KEEP COLD** because as soon as warms up coats the plastic/glass/etc.

2. Transwell Unit: Corning-Costar 3422 transwell, permeable support 12 wells plate, 8μm pore size polycarbonate membrane.
3. BSA media: DMEM, 0.1%BSA (Note: you can add P/S to the media for long-term storage).
4. Q-tips (cotton swabs), 10%formalin, Hematoxylin, Permont (Fisher).

II. Transwell Assay Using Soluble Chemoattractents

1. place 100 ul of this dilutant into inserts .
   Note: optimal concentration of Matrigel should be determined by the user, for MG63, 143B, HOS, and TE85, we use 0.2 mg/ml.

2. keep the inserts in a clean bench and let them air dry under hood and no UV light .

3. Rehydration: Add warm culture medium (200 ul) to the interior of the inserts and bottom of wells. Allow to rehydrate for 2 hours in humidified tissue culture incubator. After rehydration, carefully remove the medium without disturbing the layer of Matrigel on the membrane.

4. Prepare cell suspensions in culture medium containing 1-2 x10^5cells/ml for 24- well chambers (serum free culture medium with 0.1%BSA).

5. Add chemoattractant (300ul) to the lower wells.
Note: we usually use 10%FBS in culture media as a chemoattractant.

6. Use sterile forceps to transfer the chambers and control inserts to the wells containing the chemoattractant. Be sure that no air bubbles are trapped beneath the membranes. This can be avoided by tipping the insert or chamber at a slight angle as it is lowered into the liquid.

7. Immediately add 0.3ml of cell suspension.

8. Incubate for 24 hours in a humidified tissue culture incubator.

9. Remove insert from the chamber. Turn chamber upside-down and gently submerge in PBS several times to remove unattached cells.

10. Fix cells by submerging insert in 10% formalin for 10min. Then wash with PBS once.

11. Stain cells by soaking insert in hematoxylin for 30min ,then wash the insert gently several times with tap water to remove excess stain.

12. Removal of non-invading cells
After incubation, the non-invading cells are removed from the upper surface of the membrane by “scrubbing”. Scrubbing must be accomplished quickly to avoid drying of the cells adhering to the bottom surface of the membrane. Insert a cotton tipped swab into inserts and apply gentle but firm pressure while moving the tip over the membrane surface. Repeat the scrubbing with a second swab (Q-tip) moistened with medium.

13. Cut out membrane from insert with the migrated side down to a glass slide. Add a few drops of Permont onto the membrane and cover it with cover slide evenly.

14. Count migrated cells on a grid under high power (e.g., x20 or x30). You usually need to count 5 to 10 high power fields (HPFs) to determine the average cell number migrated per HPF.(IPP software can be used to count the cells as it is very convenient and accurate)