Matrigel Invasion Assay General Protocol
Stack Lab

1. Prepare the cells: wash the cells 3 times with PBS and culture in serum free medium (SFM) for 3 hours or o/n.
2. Prepare invasion chambers (8 micron, BD # 354578)
3. Matrigel - Dilute commercial stock to 1mg/ml (can store at -80°C). Thaw Matrigel at 4°C, add 100 ul/insert, place at RT for 1 hour. Gently take out unpermeablized liquid by using pipette. Wash once with 100 ul of SFM. Let the filter air dry (leaving the plate in hood when you prepare the cells).
4. Trypsynize the cells, neutralize the trypsin by adding equal amount of Soybean Trypsin Inhibitor. Spin down the cells. The cells are reconstituted in SFM at a final concentration of 500,000 cells/ml. Add 750 ml of SFM to the well and 500 ul of prepared cells to the insert. Avoid bubble at both sides of filter.
5. Incubate it in 37°C, 24 – 72 hours depending on cell line. [note - recommend that the initial experiment be a time course in which several samples are set up and stopped at various time points from 12-72 h]
6. Stop the assay by using Diff-quick Kit. Remove cells from the top of the filter by using a Q-Tip twice. Dip the inserts 1 min for each solution (fix solution, solution I and II). Dip the insert in the water to wash out the dye.
7. After the filter dries, remove from insert with a scalpel and mount the filter on the slide with cell sides face down using permount.
8. Count the result under microscope (20X). See file "Cell Counting Migration Invasion Assays" for cell counting protocol