Migration Assay Protocol:

Materials to be prepared beforehand:

- 1) FBS free medium
- 2) 10% FBS medium
- 3) Cell migration filter insert (Transwell<sup>®</sup>, 12mm Diameter, 12 µm Pore Size.)

Procedures:

- Coat the underside of filter inserts with 20μg/ml of fibronectin overnight at 4 °C Air dry the insert before the cells were added.
- 2) Trypsinize cells and wash twice with FBS free medium.
- 3) Count cells to  $4 \times 10^5$ /ml.
- 4) Add 0.5ml cells in FBS free medium 10 the top of insert .
- 5) Add 1ml of medium (with 10% FBS and 10 $\mu$ g/ml Collagen I ) to the lower chamber .
- 6) Put filter insert into the lower chamber and incubate overnight at  $37 \,^{\circ}$ C.
- 7) Remove cells on the topside of the filter by scrubbing twice with cotton tipped swab moistened with no FBS medium.
- 8) Stain cells with Diff-Quik<sup>TM</sup> stain kit.
- 9) Wash with distilled water.
- 10) Mount.