

Protocol for Transwell cell migration

1. The cells is washed with PBS and trypsinized prior to resuspension in serum-free in a 1.5 mL microcentrifuge tube. then cell count.
2. Add 600 μ L complete medium to the lower chamber and 300 μ L diluted cell suspension to the upper chamber.
3. Incubate for 24 h at 37 $^{\circ}$ C , 5 % CO₂.
4. The non-migrated cells were scraped off of the filter using a cotton swab
5. The cells that migrated to the lower side of the upper chamber, were fixed for 1 min with 1ml 4 % paraformaldehyde, then wash the cells in 2 washes of PBS.
6. Stain the upper chamber for 10 min. with hematoxylin, then wash the cells in 2 washes of PBS.
7. Stain the upper chamber for 1 min with eosin, transfer the upper chamber into PBS; wash the chamber with water for 5 to 15 min.
8. Dry the upper chamber in the air.