Transwell Assay

Material:

- 2% FBS in DMEM (D-2)
- 100ng/mL CCL19 in D-2
- 3um or 5um Transwell plate from Corning
- Freshly isolated CD4 T lymphocytes

Procedure:

- Prepare Transwell insert (for ICAM1 coated)
  - Dilute ICAM1 to concentration of **2ug/mL (1 tube with 400ul PBS)**
  - Put **50ul** onto each well of insert
  - Leave refrigerated overnight
  - Wash with PBS 2X before putting cells in
- Prepare cells
  - Resuspend cells in D-2 at density of **10 million/mL**
  - Rest cells in 37^{o}C water-bath until ready to put into wells
- Prepare lower chamber
  - Pipette carefully **150ul** of D-2 (as control) or CCL19 (as chemoattractant) in bottom wells, **avoid making bubbles**
  - Put **100ul** of D-2 (for total input) in separate wells
  - Take out warmed cells and vortex briefly
  - Put **50ul** of warmed cells into the 100ul of D-2 just added
  - Use a small gauge needle to **pop the bubble**s in the lower wells created while pipetting
  - Put on the insert
- Prepare top chambers
  - Briefly vortex warmed cells
  - Pipette carefully **50ul of cells** into each well except the input wells
  - **Pop the bubbles** with a small gauge needle in the top wells
  - Cover the plate with the lid
  - Incubate at 37^{o}C for 1.5 hours
- Prepare to count cells
  - Take out insert in the hood
  - Aspirate off the liquid in the insert wells
  - Use a multi-channel pipette to transfer liquid in the lower chamber to a **V-bottom plate**
  - Use **100ul of PBS** to wash the lower chamber and transfer it to the same V-bottom plate
  - Spin down briefly and stain accordingly
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