Chemotaxis Migration Protocol for Differentiated THP-1 Cells

Materials

- THP-1 cells (ATCC TIB-202)
- RPMI 1640 Medium (Life Technologies 11875-085)
- Fetal Bovine Serum (Sigma-Aldrich F2442-500mL)
- 2-Mercaptoethanol (Life Technologies 21985-023)
- Phorbol-12-myristate-13-acetate (Sigma-Aldrich 8139) - PMA
- D-PBS (w/o Ca^{2+}, Mg^{2+}, Life Technologies 10010)
- Recombinant Human C5a (Peprotech 300-70)
- IncuCyte™ ClearView™ 96-Well Cell Migration Plate (Essen 4582 or 4599)

Differentiation of THP-1 (directly within the ClearView™ insert plate)

1) Coat both sides of the insert membrane with 5 µg/mL fibronectin diluted in DPBS -/-, adding 20 µL to the insert wells (reverse pipette) and 150 µL to the reservoir wells (pre-fill reservoir and gently place the insert into the reservoir plate containing coating matrix). Incubate at ambient temperature for 1 hour.
2) Aspirate the fibronectin coating from the reservoir plate then add 200 µL D-PBS -/- to the wells and gently place the insert into the reservoir.
3) Immediately prior to THP-1 cell addition, add 40 µL DPBS -/- directly to insert wells containing fibronectin, then aspirate full volume of DPBS/fibronectin (60 µL).
4) Harvest THP-1 cells and perform a cell count (e.g., trypan blue staining + hemacytometer). Centrifuge the cell suspension (350 x g, 4 minutes) and resuspend the cell pellet in RPMI1640 + 10% FBS + 5 ng/mL PMA + 0.1% 2-ME at 41,667 cells per mL (2,500 cells per well).
   NOTE: THP-1 cells should be maintained at a cell density between 5 and 8 x 10^5 cells/mL prior to cell differentiation.
5) Allow the cells to settle at ambient temperature for 45-60 minutes then place the ClearView™ plate at 37 °C for 48 hours.

Chemotaxis Assay

1) After 48 hours, aspirate the media from the insert wells containing differentiated THP-1 cells and replace with RPMI 1640 + 0.5% FBS.
2) Using a manual multi-channel pipette, add 200 µL of the chemoattractant (for differentiated THP-1 cells we recommend C5a as a positive control) and control medium to the appropriate wells of the second reservoir plate.
3) Carefully transfer the insert plate containing the cells into the pre-filled second reservoir plate containing medium ± chemoattractant.
4) Place the IncuCyte™ ClearView™ cell migration plate into the IncuCyte ZOOM® instrument and allow the plate to warm to 37°C for at least 15 minutes. **After 15 minutes, wipe away any condensation that remains on the outside of the plate lid or bottom of the reservoir.**
5) In the IncuCyte ZOOM® software, schedule 24 hour repeat scanning (10x).
   a. Objective: Ensure 10x objective is installed
   b. Vessel Type: Select “ClearView Cell Migration”
   c. Channel Selection: Select “Phase”
   d. Scan Mode: Select “Chemotaxis (Top/Bot)” scan type and desired Scan Pattern
   e. Note the IncuCyte™ instrument estimates a scan time of 20 min per plate (phase only); however, **the actual scan time can take longer.**