SARTORIUS

Protocol

Differentiated THP-1 Incucyte® Chemotaxis Migration Protocol

Materials

- THP-1 cells (ATCC TIB-202)
- RPMI 1640 Medium (Life Technologies 11875-085)
- Fetal Bovine Serum (FBS; 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 Plate (Sartorius Cat. No. 4582, 4599, or 4648)

General Guidelines

- THP-1 cells should be maintained at a cell density between 5–8 x 10^5 cells/mL prior to cell differentiation. Maintaining THP-1 cells outside of the recommended culturing range inhibits cell migration.
- Following cell seeding, place plates at ambient temperature for 45 minutes to ensure homogeneous cell settling.
- Remove bubbles from all wells by gently squeezing a wash bottle (containing 70–100% ethanol with the inner straw removed) to blow vapor over the surface of each well.
- After placing the plate in the Incucyte® Live-Cell Analysis System, allow the plate to warm to 37 °C for 30 minutes prior to scanning.

Protocol

Differentiation of THP-1 (Directly Within the Insert of the Incucyte® Clearview Plate)

1. Prepare 5 µg/mL fibronectin diluted in D-PBS -/-.
2. Using reverse pipetting, add 150 µL of diluted fibronectin to reservoir wells of Incucyte® Clearview Plate. At a slight angle, gently lower the insert plate into the reservoir plate containing coating matrix.
3. Using reverse pipetting add 20 µL to the insert wells and incubate the Incucyte® Clearview Plate at ambient temperature for 1 hour.
4. 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.
5. Immediately prior to THP-1 cell addition, add 40 µL D-PBS -/- directly to insert wells containing fibronectin, then aspirate full volume of D-PBS/ fibronectin (60 µL).
6. 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 RPMI 1640 + 10% FBS + 5 ng/mL PMA + 0.1% 2-ME at 41,667 cells per mL.

   Note: THP-1 cells should be maintained at a cell density between 5–8 x 10^5 cells/mL prior to cell differentiation.
7. Using a manual multi-channel pipette and reverse pipetting technique, seed cells (60 µL per well, 2,500 cells per well) into every well of the insert plate. Allow the cells to settle at ambient temperature for 45–60 minutes then place the Incucyte® Clearview Plate at 37 °C for 48 hours.

Incucyte® Chemotaxis Assay

1. After 48 hours, aspirate the media from the insert wells containing differentiated THP-1 cells and replace with 60 µL 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 , EC_{50} = 86.8 nM, 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 Plate into the Incucyte® Live-Cell Analysis System and allow the plate to warm to 37 °C for at least 15 minutes.

   Note: 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® software, schedule 24 hour repeat scanning (10X).
   a. Objective: Ensure 10X objective is installed
   b. Vessel Type: Select "Incucyte® Clearview Plate"
   c. Channel Selection: Select "Phase"
   d. Scan Mode: Select "Chemotaxis (Top/Bot)" scan type and desired Scan Pattern
   e. Note the Incucyte® Live-Cell Analysis System estimates a scan time of 20 minutes per plate (phase only); however, the actual scan time can take longer.

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