SARTORIUS

Protocol

Detailed Incucyte® Chemotaxis Assay Protocol for Jurkat Cells

The following protocol is a detailed example designed to enable you to run a successful Incucyte® Chemotaxis Jurkat Migration Assay. We provide three membrane coating options which allow for either clustered or single cell migration.

Materials

- Jurkat (ATCC, TIB-152)
- Unopened bottle of RPMI 1640 (Life Technologies 11875-085)
- Fetal Bovine Serum (FBS; Sigma-Aldrich F2442)
- Matrigel® (Corning 354234)—optional
- Fibronectin (Sigma Aldrich, F1141)
- Protein G (Life Technologies 101200)—optional
- ICAM (Life Technologies 10346-H03H or Sino Biologicals Inc. 10346-H03H)—optional
- Bovine Serum Albumin (BSA; Sigma Aldrich, A7906)
- D-PBS (w/o Ca^{2+}, Mg^{2+}, Life Technologies 10010)
- SDF-1a (R&D Systems 350-NS-050)
- Incucyte® Clearview 96-well Plate for Chemotaxis (Sartorius Cat. No. 4582, 4599, or 4648)

Membrane Coating Options

Coating with Matrigel®

Clustered Cell Migration

1. Pre-cool an Incucyte® Clearview Plate in a CoolBox® system containing a frozen cold pack and CoolSink® plate (4 °C), for 5 minutes.

   Note: It is important to keep close temperature control of Matrigel® to prevent unwanted gelling. The Incucyte® Cell Invasion Kit (Sartorius Cat. No. 4444) includes a specialized CoolBox® system to ensure the temperature of your assay plate and biomatrix materials are maintained between 4–8°C—preventing premature polymerization and eliminating edge effects. Crushed ice can be used as an alternative, however non-uniform cooling can lead to assay variability.

2. On ice, prepare 50 µg/mL Matrigel® + 10% FBS diluted in pre-chilled RPMI + 0.5% FBS. Gently invert to mix.
3. Using reverse pipetting, add 150 µL of the Matrigel® solution to the chilled reservoir plate wells. At a slight angle, place the insert plate into the reservoir plate and gently roll the plate into position. Using reverse pipetting, add 20 µL of the Matrigel® solution to the insert wells.

4. Place the plate at 37 °C and incubate for 30 minutes.
5. Remove the Incucyte® Clearview Plate from 37 °C and allow to cool down to ambient temperature for 30 minutes.

   Note: This step is important in order to achieve even cell distribution.
6. Prior to cell seeding, aspirate the Matrigel® coating from the insert well and reservoir wells of the Incucyte® Clearview Plate. To the reservoir wells, aliquot 200 µL of D-PBS and gently return the insert into to the reservoir plate.

   Note: Alternatively, if removal of Matrigel® is not desired, cells can be seeded directly into the wells containing coating. The volume of cells being added to the insert must be reduced to 40 µL (refer to step 4, Chemotaxis Assay).

Coating with ICAM

Single Cell Migration

1. Resuspend Protein G and ICAM reagents in sterile water.
2. Using reverse pipetting, coat the top surface of the Incucyte® Clearview Plate membrane with 20 µL of 20 µg/mL Protein G solution and incubate for 1 hour at 37 °C.
3. Wash the membrane once with D-PBS by adding 40 µL D-PBS directly to the wells containing Protein G. Remove the full volume (~ 60 µL) and promptly proceed with the ICAM coating step.
4. Using reverse pipetting, add 20 µL of 5 µg/mL ICAM to the insert wells and incubate for 2 hours at 37 °C.
5. Aspirate ICAM from the insert, then block both sides of the membrane with D-PBS + 1% BSA by adding 20 µL to the insert wells and 150 µL to the reservoir wells of the Incucyte®Clearview Plate (pre-fill reservoir and gently place the insert into the reservoir plate containing BSA). Incubate at ambient temperature for 30 minutes.
6. After incubation, transfer the insert plate to a new Incucyte® Clearview Reservoir Plate containing 200 µL D-PBS in each well. Immediately prior to cell addition, wash the insert wells once with D-PBS by adding 40 µL D-PBS to the insert wells containing 1% BSA, then remove the full volume (~ 60 µL).

Coating with Fibronectin

Single Cell Migration

1. Prepare fibronectin at 5 µg/mL in D-PBS (without calcium or magnesium) supplemented with 0.1% BSA.
2. Using reverse pipetting, add 150 µL of fibronectin solution into the reservoir of the Incucyte® Clearview Plate. Place the insert into the reservoir and, using reverse pipetting, add 20 µL of the fibronectin solution into the insert. In this case, a second reservoir plate will be loaded with chemoattractant and used during the experiment.
3. Incubate for 1 hour at ambient temperature.
4. Aspirate the fibronectin + 0.1% BSA coating from the reservoir wells and replace with 200 µL of D-PBS and gently return the insert into to the reservoir plate.
5. To the insert, add 60 µL of D-PBS to the wells containing fibronectin + 0.1% BSA, then aspirate immediately prior to cell seeding.

Protocol

Incucyte® Chemotaxis Assay

1. Thaw Jurkat cell line and wash 1X in 5 mL of serum free RPMI 1640 media.
2. Centrifuge cells at 500 x g for 5 minutes.
3. Re-suspend cells in an appropriate volume of chemotaxis assay media (newly opened RPMI + 0.5% FBS) and perform a cell count.
4. Using a manual multi-channel pipette and reverse pipetting technique, seed cells (60 µL per well, 5,000 cells per well) into every well of the insert plate of the Incucyte® Clearview Plate.

   Calculation: 83,333 cells/mL x 0.06 mL = 5,000 cells per insert well.
5. Allow the Jurkats to settle at ambient temperature on a level surface for 45–60 minutes.
6. During cell settling, prepare chemoattractant dilutions and controls (for reference, standard range of SDF-1α is 12.5–125 nM).
7. Using a manual multi-channel pipette, add 200 µL of the chemoattractant and control medium to the appropriate wells of the second reservoir plate.
8. Carefully transfer the insert plate containing the cells into the pre-filled second reservoir plate containing medium ± chemoattractant.
9. 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.
10. In the Incucyte® software, schedule 24 hour repeat scanning (10X) for every 30 minutes.

    Note: This schedule is only for a scanning a single plate. Fewer scans will be required if scheduling multiple plates.
    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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