Chemotaxis Migration Detailed Demonstration 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)
- Lympholyte-H (Cedarlane CL5010)
- RPMI 1640 (Life Technologies 11875-085)
- Fetal Bovine Serum (Sigma-Aldrich F2442)
- Matrigel® (Corning 354234) – optional
- Fibronectin (Sigma Aldrich, F1141)
- Protein G (Life Technologies 101200) – optional
- ICAM (Life Technologies 10346-H03H) – optional
- Bovine Serum Albumin (BSA; Sigma Aldrich, A7906)
- D-PBS (w/o Ca^{2+}, Mg^{2+}, Life Technologies 10010)
- SDF-1a (RnD systems 350-NS-050)
- IncuCyte™ ClearView™ 96-Well Cell Migration Plate (Essen 4582 or 4599)

Membrane Coating Protocols

Coating with Matrigel

Clustered Cell Migration

1) Coat both sides of the membrane with 50 µg/mL Matrigel diluted in RMPI media + 10% FBS by 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). In this case, a second reservoir plate will be loaded with chemoattractant and used during the experiment.
   NOTE: The chemotaxis plate, must be pre-chilled to 4°C. We recommend using a CoolSink to keep the plate cold during the coating procedure.
2) Place the plate at 37°C and incubate for 30 minutes.
3) Remove the Transmembrane 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.
4) Prior to cell seeding, aspirate the Matrigel® coating from the insert well and reservoir wells. To the reservoir, aliquot 200 µL of DPBS 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) Coat the top of the membrane with 20 µL of 20 µg/mL Protein G solution for 1 hr at 37°C. Remember to use reverse pipetting when adding volume to the insert wells.
2) Wash the membrane once with PBS. To wash, add 40 µL PBS to the Protein G–containing insert wells. Then remove the full volume (~60 µL) and promptly proceed with the ICAM coating step.
3) Next, coat the top of the membrane with 20 µL of 5 µg/mL ICAM for 2 hr at 37 °C.
4) Block both sides of the membrane with PBS + 1% BSA: 20 µL on top and 150 µL in the reservoir. Incubate for 30 min at ambient temperature.
5) After incubation, transfer the insert plate to a new reservoir with 200 µL PBS in each well. Immediately prior to cell addition, wash the insert wells once with PBS as described above.

Coating with Fibronectin

Single Cell Migration

1) Prepare fibronectin at 5 µg/ml in PBS (without calcium or magnesium) supplemented with 0.1% BSA.
2) Pipette 150 µl of fibronectin solution into the reservoir. Place the insert into the reservoir and pipette 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.
   NOTE: To reduce bubbles a reverse pipetting technique should be used for all liquid transfers into reservoirs and inserts.
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 DPBS and gently return the insert into to the reservoir plate.
5) To the insert, add 60 µL of DPBS to the wells containing fibronectin + 0.1% BSA, then aspirate prior to cell seeding.

Chemotaxis Assay

1) Thaw Jurkat cell line and wash 1x in 5 mL of serum free RPMI 1640 media.
2) Centrifuge cells at 500xg for 5 minutes.
3) Re-suspend cells in an appropriate volume of chemotaxis assay media (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.
   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.
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™ 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.
10) In the IncuCyte ZOOM® software, schedule 24 hour repeat scanning (10x) for every 30 minutes.
    NOTE: This schedule is only for a scanning a single plate. Fewer scans times will be required if scheduling multiple plates.
    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.**