SARTORIUS

Protocol

Incucyte® Chemotaxis Cell Invasion Assay

For the Detection of Chemotactic-Induced Cell Invasion

The Incucyte® Chemotaxis Cell Invasion Assay approach enables automated imaging and analysis of cell invasion using an optically clear membrane that allows for 96-Well kinetic throughput. Measurements of chemotactic cell invasion can be made using nuclear labeled or unlabeled cells, however, we recommend labeling cells with a live-cell nuclear label (e.g., Incucyte® Nuclight Reagents) to ensure optimal performance. Using Incucyte® integrated metrics, we are able to precisely quantify the chemotactic response of adherent types, with direct visualization of cell invasion.

Required Materials

- Incucyte® Clearview 96-Well Plate (Sartorius Cat. No. 4582)
- Incucyte® Clearview Reservoir Plate (Sartorius Cat. No. 4600)
- Incucyte® Cell Invasion Kit (Sartorius Cat. No. 4444)
- Cultrex® 3-D Culture Matrix™ Reduced Growth Factor Basement Membrane Extract (Trevigen Cat. No. 3445-005-01)—optional
- Cultrex® Rat Collagen I (Trevigen Cat. No. 3440-100-01)—optional
- Incucyte® Chemotaxis Analysis Software Module (Sartorius Cat. No. 9600-0015)

General Guidelines

Avoiding Bubbles

The Incucyte® Live-Cell Analysis System relies on images to process data; thus, it is important to avoid bubbles and follow our protocol recommendations to achieve superior assay performance and imaging. We recommend the following techniques to eliminate bubbles from your experiment:

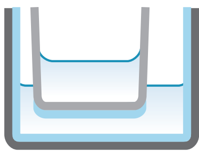
- Reverse-pipette at the coating step and when adding cells to the insert. Reverse pipetting reduces the risk of splashing or bubble formation. In reverse pipetting, the volume aspirated into the tip is larger than the volume delivered to the receiving vessel.
  - Press the plunger to the second stop.
  - Dip the pipette-tip into the solution.
  - Release the plunger until the starting has been reached.
  - Move the pipette-tip to the receiving vessel.
  - Dispense the liquid by pressing the plunger to the first stop. Some liquid will remain in the tip.
  - Repeat steps 2–5 throughout the plate.
- Triturate with an additional cell volume or reduced volume setting (e.g., 60 µL cell volume added, mix by reverse-pipetting up and down with 30 µL) to dislodge bubbles that may have been trapped at the membrane-insert interface. Perform this immediately after cell addition.
- Remove bubbles at the liquid surface by gently squeezing a wash bottle containing 100% ethanol, with the inner straw removed, to blow vapor over the surface of each well.

Reagent Temperature Control

- It is important to keep close temperature control of biomatrix materials such as Collagen-1 and basement membrane extract (BME) to prevent unwanted gelling.
- The Incucyte® Cell Invasion Kit (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.
- When handling cells that have been embedded in biomatrix material, ensure that all steps of cell handling is performed between 4–8, utilizing consumables that have been pre-chilled (e.g., a pre-cooled reservoir boat during cell seeding).

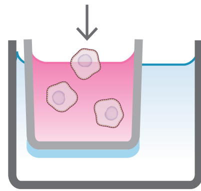
Protocol

1. Prime insert



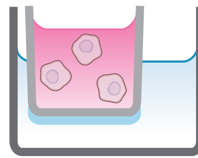
Prime membrane by adding 150 µL of D-PBS to the reservoir of a pre-chilled Clearview Plate. Incubate plate for 20 minutes at 4° C.

2. Harvest and seed cells



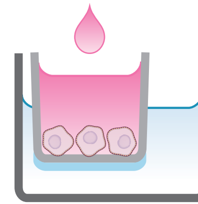
Prepare cells at 50,000 cells/mL in extracellular matrix. Dispense 20 µL/well of cell:matrix suspension (1,000 cells/well) into insert. Centrifuge for 3 minutes at 50 x g.

3. Polymerize matrix



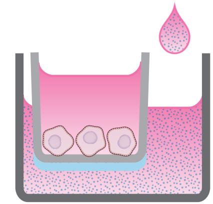
Place Clearview Plate on a pre-warmed CoolSink at 37° C. Allow matrix with embedded cells to polymerize for 30–60 minutes.

4. Media addition



Add 40 µL assay medium on top of the polymerized matrix: cell layer.

5. Add chemoattractant



Add 200 µL of desired chemoattractant or controls to appropriate wells of the reservoir plate. Place the insert into the pre- filled and image in Incucyte® Live-Cell Analysis System.

Day 0

Prime Insert

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

1.2 Add 150 µL of D-PBS (4° C) to each reservoir well of the pre-chilled Incucyte® Clearview Plate. Replace the Incucyte® Clearview insert and allow the membrane to prime for 20 minutes within the CoolBox.

Harvest and Seed Cells

2.1 While the Incucyte® Clearview Plate is priming, prepare your biomatrix reagent at the desired working concentration as per the manufacturer's instructions. Prepare a large dead volume to ensure available biomatrix for transfer to the assay plate (e.g., prepare 4 mL biomatrix solution to provide 20 µL per insert well).

Note: The required biomatrix density will be dependent on the matrix and cell types used. For HT-1080 cells we recommend BME (5 mg/mL) diluted in assay medium or Collagen I (1 mg/mL) diluted in neutralizing buffer (DMEM, Sigma Cat. No. D2429, +7.5 g/L sodium bicarbonate + 0.004 g/L folic acid + 1% GlutaMax). When preparing collagen I it is important that it is properly neutralized to ensure cell health is maintained and gelling is uniform.

2.2 Harvest your cells and resuspend the pellet in the biomatrix solution. Cell density will need to be optimized for each cell type used; however, we have found that 50,000 cells/mL (1,000 cells per well) is a reasonable starting point.

Calculation: 50,000 cells/well x 0.02 mL/well = 1,000 cells/well.

Note: Some cell types may require reduced exposure to Fetal Bovine Serum (FBS) before initiating the transmembrane invasion assay (e.g., HT-1080s starved in F12 + Insulin- Transferrin-Selenium for ~ 20 hours).

Polymerize Matrix

3.1 Place the Incucyte® Clearview Plate at 37° C on a pre-warmed CoolSink and allow the biomatrix to polymerize for 30–60 minutes.

Media Addition

4.1 Gently add 40 µL of assay medium ± modulators of invasion on top of the biomatrix:cell layer in the insert wells.

Note: If adding modulators of invasion, the working concentration should be 1.5X the desired final concentration to account for the volume of the biomatrix: cell layer

Chemoattractant Addition and Imaging

5.1 Add 200 µL of desired chemoattractant or control to the appropriate wells of a second Incucyte® Clearview reservoir plate.

5.2 Carefully transfer the insert into the preloaded reservoir plate. Be careful not to introduce bubbles which can become trapped below the membrane when placing the insert into the pre-filled reservoir plate.

5.3 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. After 15 minutes, carefully wipe away any condensation that may have accumulated on top of the plate lid or on the bottom of the reservoir plate. Schedule 24 hour repeat scanning:

a. Objective: 10X
b. Channel selection: Phase Contrast + Fluorescent channels selected (dependent on the fluorescent label used)
c. Scan type: Chemotaxis
d. Scan interval: Every 1 to 2 hours

Sales and Service Contacts

For further contacts, visit www.sartorius.com

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