SARTORIUS

Protocol

Incucyte® Chemotaxis Cell Migration Assay

For the Detection of Chemotactic-Induced Cell Migration

The Incucyte® Chemotaxis Cell Migration Assay approach enables automated imaging and analysis of cell migration using an optically clear membrane that allows for 96-well kinetic throughput. Using phase-contrast and | or fluorescent imaging in combination with Incucyte® integrated metrics, we are able to precisely quantify the chemotactic response of adherent or non-adherent cell types. The assay method does not require labeling for quantification, is sensitive to surface integrin signaling, sustains a linear gradient over several days and allows for direct visualization of cell migration.

Required Materials

- Incucyte® Clearview 96-Well Plate (Sartorius Cat. No. 4582)
- Incucyte® Clearview Reservoir Plate (Sartorius Cat. No. 4600)
- Incucyte® Chemotaxis Analysis Software Module (Sartorius Cat. No. 9600-0015)

General Guidelines

It is important to read the protocol in its entirety prior to initiating a chemotaxis assay. 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 position 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 until 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.
- Gently place the insert into the pre-filled reservoir at a slight angle to allow air to move upwards across the membrane.

Optimization Considerations

Cells in the Incucyte® Chemotaxis Cell Migration Assay are required to move toward a chemoattractant gradient across the membrane surface and through a pore. As a result, cells must be maintained in a healthy state on a biologically relevant surface, in order to facilitate cell movement. Cell surface coatings, chemoattractant, and assay medium formulations are parameters that should be optimized in order to achieve superior assay performance. Prior to starting optimization assays, we recommend a thorough review of literature in order to become familiar with standard culturing techniques as well as Boyden chamber assay conditions for the cell type of interest. Although not all techniques will translate into the Incucyte® Chemotaxis Cell Migration Assay, they will give guidance for an optimization strategy.

Coating: Migrating cells require interactions with the substrate in order to move. Collagen I, collagen IV, gelatin (Attachment Factor), fibronectin, Matrigel®, and protein G/ICAM surface coatings are commonly used to promote cell migration. Refer to "Incucyte® Clearview Plate Coating Protocol" for recommended coating procedures.

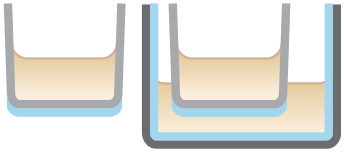
Chemoattractant: The chemoattractant concentration required for an optimal assay should be determined through experimentation. Based on published literature and/or experience, we recommend testing the expected EC_{50} concentration as well as testing concentrations 1–2 logs above and below the EC_{50}.

Medium: We have found that including a reduced amount of serum (0.1%–2.5%) in the assay buffer allows the cells to attach to the surface and move, while not affecting their directional migration. If cells look unhealthy, an experiment should be designed to increase serum or growth factor levels until cells are healthy enough to attach and move. Adding Insulin-Transferrin-Selenium (ITS) to the assay medium is another way to make cells healthier if minimizing serum is required.

Protocol

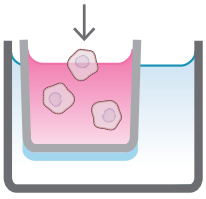
Quick Guide

1. Coat insert (optional)



Coat membrane with extracellular matrix (ECM): 20 µL (insert side) and 150 µL (reservoir side).

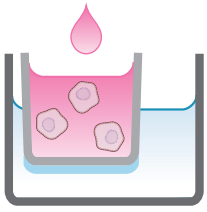
2. Harvest and seed cells



Seed cells (*60 µL/well, 1,000 or 5,000/well) into the Incucyte® Clearview insert.

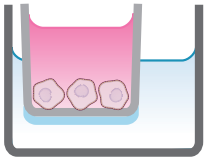
*If assay requires addition of treatment, seeding volume should be reduced to 40 µL.

3. Treat cells (optional)



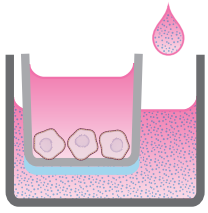
Prepare 3X concentrations of treatment and add 20 µL to appropriate wells. Mix by triturating a 30 µL volume.

4. Allow cells to settle



Place on level surface and allow cells to settle at ambient temperature for 15 to 60 minutes.

5. Add chemoattractant



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

Day 0

Coat insert (optional)

Some cell lines may require the addition of an extracellular matrix protein (e.g., 5 µg/mL fibronectin) to promote light cell adherence and provide the necessary integrins for cell motility. Refer to Table 1 for cell-line specific coating recommendations.

1.1 Under sterile conditions prepare coating matrix at desired concentration.

1.2 Using reverse pipetting, aliquot 150 µL of the prepared matrix into the Incucyte® Clearview reservoir wells and 20 µL into the insert wells.

1.3 Incubate according to manufacturer's recommendations.

1.4 If required, aspirate and wash coating from the reservoir and insert prior to cell seeding.

Harvest and Seed Cells

2.1 Harvest cells using suitable dissociation solution.

2.2 Spin down cells and resuspend in appropriate assay medium, i.e., growth medium with reduced FBS, typically 0.5% FBS.

2.3 Determine cell concentration and prepare cell seeding stock to achieve 1,000 or 5,000 cells per well at a 60 µL volume, or, if using modulators of chemotaxis (refer to step 3 below), the seeding volume will be reduced to 40 µL.

Note: The seeding density will need to be optimized for each cell type used; however, we have found that 1,000 cells per well for adherent cell types and 5,000 cells per well for non-adherent cell types are reasonable starting points.

2.4 Using reverse pipetting, add cells to Incucyte® Clearview insert wells (if not using inhibitors of chemotaxis, go directly to step 4).

Seeding Volume	Final Cell Density/ Well	Cell Seeding Stock (Cells/mL)	Recommended Seeding Stock Volume (mL)
40	1,000	25,000	6
40	5,000	125,000	5
60	1,000	16,666.70	8
60	5,000	83,333.30	8

Treat Cells (optional)

Some cell lines may require the addition of an extracellular matrix protein (e.g., 5 µg/mL fibronectin) to promote light cell adherence and provide the necessary integrins for cell motility. Refer to Table 1 for cell-line specific coating recommendations.

3.1 Prepare 3X concentrations of treatment and add 20 µL to the Incucyte® Clearview insert wells containing cells immediately after cell seeding.

3.2 Using a 30 µL volume, triturate the cells to appropriately mix the treatment, so cell exposure during pre-treatment is at 1X.

Harvest and Seed Cells

4.1 Place the plate onto a level surface and allow the cells to settle at ambient temperature for 15 minutes (adherent cell types) to 45–60 minutes (non-adherent cell types).

Note: If treatments were added to an adherent cell type, we recommend a continued pre-incubation with inhibitors at 37° C for 30 minutes.

Chemoattractant Addition and Imaging

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

5.2 Carefully transfer the Incucyte® Clearview insert into the pre-loaded 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 (+ Fluorescence if fluorescent labeled cell line is used)

c. Scan type: Chemotaxis

d. Scan interval: Every 1 to 2 hours

	Therapeutic Area	Cell Type	Assay Medium	Coating (20 µL Top/ 150 µL Bottom)	Seed Density (Cells/Well)	Cell Setting Time (Min)	Chemoattractant (Concentration Range Tested) nM	ng/ML
Validated Cell Lines (Non-Adherent)	Immunology	Jurkat	RPMI + 0.5% FBS	5 µg/mL Fibronectin + 0.1% BSA in D-PBS 50 µg/L Matrigel + 10% FBS in assay medium (for clustered cell migration)	5,000	45–60	SDF-1α (12.5–125)	SDF-1α (100–1,000)
	Immunology	T Cells	RPMI + 0.5% FBS	20 µg/mL Protein G followed by 5 µg/mL ICAM	5,000	45–60	SDF-1α (3.1–200) CXCL11 (12.5–800 RANTES (16)	SDF-1α (24.8–1,600) CXCL11 (103.8–6,640) RANTES (125)
	Immunology	Neutrophils	RPMI + 0.5% HSA	50 µg/mL Matrigel + 10% FBS in assay medium	5,000	45–60	C5α (2.6–1,900) fMLP (1.4–1,000) IL-8 (1.5–1120) LTB4 (1.4–1,000)	C5α (21.6–15,770) fMLP (0.6–437.6) IL-8 (13.4–10,000) LTB4 (0.5–336.5)
	Immunology	CCRF-CEM	RPMI + 50% Human Serum	20 µg/mL Protein G followed by 5 µg/mL ICAM OR 5 µg/mL Fibronectin	5,000	45–60	CCL22 (1–100) SDF-1α (1–100)	CCL22 (8.1–810) SDF-1α (8–800 nM)
	Immunology	THP-1	RPMI + 0.5% dialyzed FBS	50 µg/mL Matrigel + 10% (dialyzed FBS in assay medium)	5,000	45–60	RANTES (08–780) MIP-1α (0.8–780) MCP-1 (12–8,600)	RANTES (0.1–100) MIP-1α (0.1–100) MCP-1 (1.4–1,000)
	Immunology	Differentiated THP-1	RPMI + 0.5% FBS	5 µg/mL Fibronectin in D-PBS	2,500	45–60**	C5a (0.5–2,000)	C5a (4.2–16,600)
	Immunology	Primary Macrophages	RPMI + 0.5% FBS	50 µg/mL Matrigel + 10% FBS in assay medium	2,500	15–30	C5a (10–1000)	C5a (83–8,300)
Validated Cell Lines (Adherent)	Vascular	HUVEC	EBM-2 + ITS + 0.25% FBS with #supplements	5 µg/mL Fibronectin + 0.1% BSA in D-PB	1,000	15	VEGF (0.01–2.6) bFGF (0.02–17.4) FBS (0.4 -10%)	VEGF (0.4–100) bFGF (0.4–300) FBS (0.4–10%)
	Vascular	HMVEC	EBM-2 + 0.5% FBS + ITS with #supplements	10 µg/mL Fibronectin + 0.1% BSA in D-PB	1,000	15	bFGF (0.06–5.8) FBS (10%)	bFGF (0.01-0.1) FBS (10%)
	Oncology	HT 1080	F12 + ITS*	None	1,000	15	FBS (0.01–10)%	
	Oncology	MDA-MB-231	DMEM + 2.5 FBS	None	1,000	15	EGF (0.2–1.6) FBS (10%) -slight response	EGF (1–10n) FBS (10%) slight response
	Oncology	MCF10a	DMEM/F12 + 10 µg/mL insulin hydrocortozone + 100ng/mL cholera toxin	5 µg/mL Collagen IV in 0.05M HCl	1,000	15	EGF (0.2–17,200)	EGF (0.1–1,000)

* Insulin-Transferrin-Selenium (Life Technologies Cat. No. 41400-045) settle at ambient temperature for 15 minutes (adherent cell types) to 45–60 minutes (non-adherent cell types)

Supplements: EGM-2 singlequot bullet kit (LONZA Cat. No. 4176) with growth factors removed and reduced FBS

** Cell settling time pre-differentiation

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