Cell migration is an essential component of most biological processes, including immune responses, blood vessel formation, tumor metastasis, and wound healing. The dominant solutions for monitoring cell migration include the Boyden-chamber, and microfluidic assay. Commercially available Boyden-chamber assays allow for high-throughput (96-well format), while microfluidic assays provide the ability to visualize the cell migration process. We have developed a new methodology, the IncuCyte™ Chemotaxis Cell Migration Assay, which combines the advantages of microfluidic and Boyden-chamber approaches without the associated liabilities, such as non-linear chemotactic gradients, laborious assay steps and difficult quantification. The IncuCyte™ Chemotaxis Cell Migration Assay method utilizes the IncuCyte ZOOM® instrument, a fully automated imaging system with integrated software analysis tools, and the IncuCyte™ ClearView 96-Well Cell Migration Plate consisting of, an optically clear membrane that allows for direct visualization of cell migration using phase-contrast and/or fluorescence imaging. Key advantages of the IncuCyte™ chemotaxis assay approach are 96-well throughput and the ability to visualize the cell migration process kinetically, while maintaining precision. Integrated metrics precisely quantify the chemotactic response using 1,000 to 5,000 cells per well. The assay method does not require labeling for quantification, is sensitive to surface-integrin signaling and sustains a linear gradient over several days.

**IncuCyte™ Chemotaxis Cell Migration General Protocol**

Briefly, cells (labeled or unlabeled) are added to the top chamber of the ClearView plate and allowed to settle at ambient temperature with or without compound pre-treatment. Chemoattractant is added to the reservoir plate. The insert plate is then placed into the reservoir plate, covered with the plate lid and placed into the IncuCyte ZOOM® instrument. Automated, time-lapse imaging of each membrane is performed by the instrument and integrated analysis tools are used to quantify cell migration in real time. This protocol can be used to measure differential responses toward various chemotactic factors as well as the modulation of such responses by small molecules, antibodies and/or surface integrins.

**IncuCyte™ Chemotaxis Cell Migration Assay General Protocol**

This protocol provides an overview of the IncuCyte™ Chemotaxis Cell Migration Assay methodology, which can be used with adherent or non-adherent cell types. This protocol is compatible with the IncuCyte ZOOM® instrument using nuclear-labeled or non-labeled cells.

**IncuCyte™ Chemotaxis Cell Migration Assay General Protocol**

- **1. Coat Insert (optional)**
  Coat membrane with extracellular matrix (ECM): 20 µL (insert-side) and 150 µL (reservoir-side). Note: The concentration and type of ECM will have to be optimized for each cell line used, as well as whether a top-side only coating or a top and bottom-side coating is required.

- **2. Harvest & Seed Cells**
  Seed cells (*60 µL/well, 1,000 or 5,000/well, ± coating matrix) into the IncuCyte™ ClearView 96-well Insert. Note: If assay requires addition of treatment to inhibit cell motility, 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 plate on level surface and allow cells to settle at ambient temperature for 15 to 60 minutes.

- **5. Add Chemo-attractant**
  Add 200 µL of desired chemoattractant or controls to appropriate wells of the reservoir plate. Place the insert into the pre-filled plate, being careful to avoid trapping air under the membrane.
Best Practices for Avoiding Bubbles

Air bubbles trapped on either side of the membrane can interfere with proper focusing and image processing. 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.
  1. Press the plunger to the second stop.
  2. Dip the pipette-tip into the solution.
  3. Release the plunger until the starting position has been reached.
  4. Move the pipette-tip to the receiving vessel.
  5. Dispense the liquid by pressing the plunger to the first stop. SOME LIQUID WILL REMAIN IN THE TIP.
  6. 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.

General Protocol

1) Seed cells of interest at 60 µL per well (40 µL per well if adding inhibitors of cell motility; refer to step 2) at an appropriate density into a ClearView 96-well insert. Typically this is done with the insert mated to an empty reservoir plate. 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.

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

b. Some cell lines (e.g., neutrophils) may require the addition of a basement membrane extract (e.g., 50 µg/mL Matrigel + 10% FBS) to promote light cell adherence and provide the necessary integrins for cell motility. Follow the manufacturer’s recommendations for coating. Refer to Table 1 for cell line seeding density and coating recommendations.

2) If pre-treating cells, prepare 3x treatments and immediately add 20 µL to the insert wells containing cells immediately after cell seeding. Triturate the cells, using a 30 µL volume, to appropriately mix the treatment, so cell exposure during pre-treatment is at 1x. NOTE: It is important to immediately add treatments to insert wells immediately after seeding. If adherent cells attach prior to trituration, uneven cell distribution can occur.

3) Place the plate onto a level surface and allow the cells to settle at ambient temperature for 15 minutes (adherent cell types) to 60 minutes (non-adherent cell types). For adherent cell types, we recommend a continued pre-incubation with inhibitors at 37°C for 30 minutes.

4) Add 200 µL of desired chemoattractant or control to the appropriate wells of a second reservoir plate. Carefully transfer the 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) Place the 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, carefully wipe away any condensation that may have accumulated on the plate lid or bottom of the reservoir.

6) Image the plate in the IncuCyte ZOOM® instrument with a 10x objective using the Chemotaxis Scan Type.
<table>
<thead>
<tr>
<th>Therapeutic Area</th>
<th>Cell Type</th>
<th>Assay Medium</th>
<th>Starvation Time (hr)</th>
<th>Coating (20 µL top/150 µL bottom)</th>
<th>Cell Settling Time (min)</th>
<th>Seeding Density (cells/well)</th>
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<tbody>
<tr>
<td>Non-adherent</td>
<td>Jurkat</td>
<td>RPMI + 0.5% FBS</td>
<td>None</td>
<td>5 µg/mL Fibronectin + 0.1% BSA in D-PBS</td>
<td>45-60</td>
<td>5,000</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>50 µg/mL Matrigel + 10% FBS in assay medium (for clustered cell migration)</td>
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<td></td>
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<tr>
<td></td>
<td>T Cells</td>
<td>RPMI + 0.5% FBS</td>
<td>None</td>
<td>50 µg/mL Matrigel + 10% FBS in assay medium</td>
<td>45-60</td>
<td>5,000</td>
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<td></td>
<td></td>
<td></td>
<td>20 µg/mL Protein G followed by 5 µg/mL ICAM</td>
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<tr>
<td></td>
<td>Neutrophils</td>
<td>RPMI + 0.5% HSA</td>
<td>None</td>
<td>50 µg/mL Matrigel + 10% FBS in assay medium</td>
<td>45-60</td>
<td>5,000</td>
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<tr>
<td>Vascular</td>
<td>HUVEC</td>
<td>EBM-2 + ITS + 0.25% FBS</td>
<td>None</td>
<td>5 µg/mL Fibronectin + 0.1% BSA in D-PBS</td>
<td>15</td>
<td>1,000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>With supplements#</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>HMVEC</td>
<td>EBM-2 + 0.5% FBS + ITS with supplements #</td>
<td>None</td>
<td>10 µg/mL Fibronectin + 0.1% BSA in D-PBS</td>
<td>15</td>
<td>1,000</td>
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<tr>
<td>Adherent</td>
<td>HT 1080</td>
<td>F12 + ITS*</td>
<td>~20 hr</td>
<td>None</td>
<td>15</td>
<td>1,000</td>
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<tr>
<td>Oncology</td>
<td>MDA-MB-231</td>
<td>DMEM + 2.5% FBS</td>
<td>None</td>
<td>None</td>
<td>15</td>
<td>1,000</td>
</tr>
<tr>
<td></td>
<td>MCF10a</td>
<td>DMEM/F12 + 10 µg/mL insulin + 0.5 µg/mL hydrocortisone + 100 ng/mL cholera toxin</td>
<td>None</td>
<td>5 µg/mL Collagen IV in 0.05M HCl</td>
<td>15</td>
<td>1,000</td>
</tr>
</tbody>
</table>

* Insulin-Transferrin-Selenium (Life Technologies Cat# 41400-045)
# Supplements include gentamycin, hydrocortisone, ascorbic acid