IncuCyte® Chemotaxis System:

A New and Enabling Solution for Directional Migration Assays

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Summary and Impact

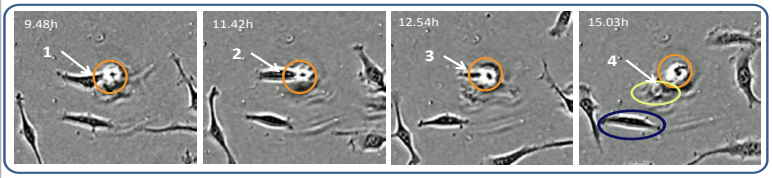
- The Transwell® Boyden chamber has been the mainstay *in vitro* method for measuring directional migration. However, it is widely acknowledged as technically tricky, hard to troubleshoot and frequently yields variable data.
- Essen Bioscience's new IncuCyte® Chemotaxis System provides a robust walkaway, fully kinetic and image-based solution in a 96-well format.
- The novel ClearView 96-well plate consumable has an ordered array of 8 µm pores created on a viewing surface in each well.
- Directional cell migration across the surface and toward chemoattractant placed in the reservoir of the plate is visualised over time using IncuCyte® live cell imaging and quantified with IncuCyte® Chemotaxis Cell Migration image analysis software.
- This integrated solution is validated for both adherent and non-adherent cell types.
- Key benefits include (1) full visualisation of the cell biology, (2) easier workflows, (3) low cell usage, (4) highly reproducible 96-well data and (5) relevant surface biology.

IncuCyte® ClearView 96-Well Cell Migration Plate

1. Coat membrane surface (optional) with extracellular matrix 20 µl (insert), 150 µl (reservoir).
2. Harvest & seed cells (60 µl, 1000 - 5000/well) into the IncuCyte™ ClearView 96-well Insert.
3. If testing compounds in insert, add treatments to appropriate wells and mix (adjust seeding volume accordingly).
4. Place plate on level surface and allow cells to settle at ambient temperature for 15-60 min.
5. Add 200 µl of desired chemoattractant or controls to appropriate wells of the reservoir plate.

1. Place membrane insert into pre-filled reservoir plate.
2. Place in IncuCyte ZOOM®
3. Capture images every 30-120 min (10x) for 1-2 days.

Visualise and Analyse Chemotaxis in Real-Time



Visualise cell migration (top panel).

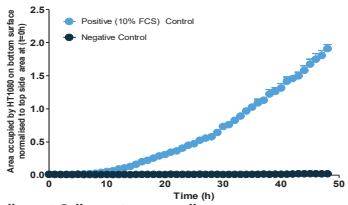
- Time-lapse images of a HT1080 cell migrating towards a pore (orange ring) and chemoattractant 10% FCS (1&2).
- The cell migrates through the pore (3) and then appears on the underside of the membrane (4).
- Cells on top surface (yellow) and cells on the underside (blue) are imaged and analysed in real-time.

Automated image analysis (bottom panel).

- High definition phase contrast images are acquired on both the top and bottom surface of the membrane.
- Automated image processing separates cells located on the top surface (outlined in yellow) and the bottom surface (outlined in blue) of the membrane. Pores are outlined in orange.
- Images are processed as they are acquired, and data can be plotted in real-time.

Real-Time Kinetic Quantification

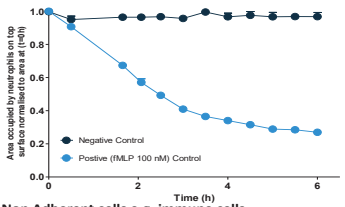
Measure Cell Appearance On Bottom Surface



Adherent Cells e.g. tumour cells.

- Data shows the response of HT1080 cells (1000/well) to 10% FCS over 48 h, images captured every 2 h.
- Adherent cells migrate across the membrane, through the pores and then remain attached to the bottom surface of the membrane.
- Measure the loss of surface area coverage by cells on the top surface or the gain of cell area coverage on the bottom surface (shown).

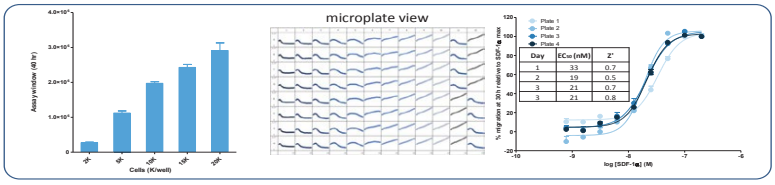
Measure Cell Loss From Top Surface



Non Adherent cells e.g. immune cells.

- Data shows the response of neutrophils (5000/well) to fMLP (100 nM) over 6 h, images captured every 30 min.
- Non-adherent cells migrate across the membrane, through the pores and then drop into the reservoir plate.
- Measure the loss of surface area coverage by cells over time.

Reproducible 96-well Assays Suitable for Screening

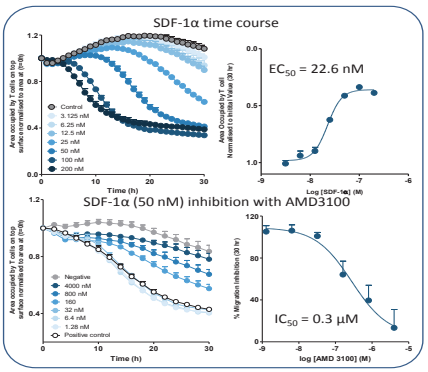


Day	EC_{50} (nM)	Z'
1	33	0.7
2	19	0.5
3	21	0.7
3	21	0.8

Robust 96-well assay.

- Cell density titrations illustrates low cell usage in the assay (assay run at 5K/well).
- Representative 96-well microplate graph showing Jurkat migration towards the chemoattractant SDF-1α (serial dilutions of chemoattractant across the plate).
- Z' values ranged from 0.5 to 0.7 for four replicate plates over three days.
- Corresponding concentration-dependent response curves to SDF-1α provided reproducible measurements of SDF-1α potency (EC_{50} value range 19 to 33 nM) within and between days.

Measure Pro-Anti-Migration Effects



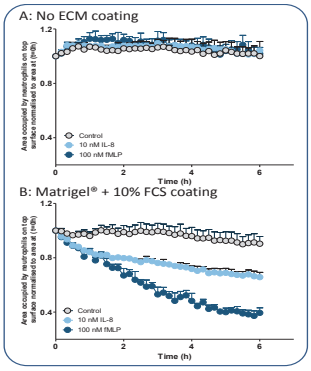
Primary T cell CXCR4 pharmacology.

- Isolated human primary T cells (5K cells/well) were seeded onto a Matrigel® (50 µg/ml) coated ClearView plate and the pharmacological response measured through the endogenous CXCR4 receptor over 30 h.
- Data (**top panel**) illustrates the time course and concentration dependant response to the CXCR4 agonist SDF-1α.
- The response with SDF-1a (50 nM, **bottom panel**) can be inhibited with AMD3100, a known CXCR4 antagonist with an IC_{50} of 0.3 µM.

Migration Across Relevant 2D Surface

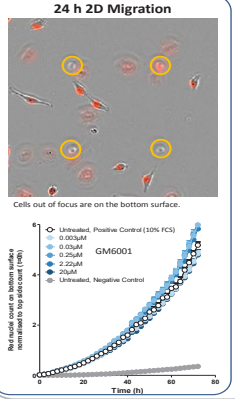
Measure relevant surface contact-mediated cell migration.

- The low pore density of the ClearView membrane requires cells to migrate across the membrane surface towards the chemoattractant.
- Neutrophils seeded on an uncoated ClearView membrane were unable to migrate towards the chemoattractants IL-8 and fMLP (**A**).
- However, those on Matrigel®-coated membranes showed clear chemotactic profiles (**B**).
- These data suggest that integrin and/or cell surface receptor interactions with the substrate play a key role in neutrophil chemotaxis in this model.

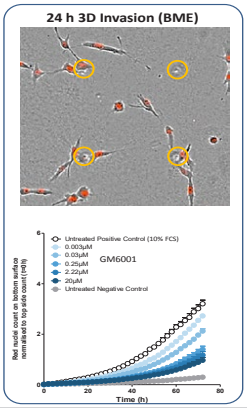


Invasion Through 3D Biomatrix

24 h 2D Migration



24 h 3D Invasion (BME)



Use of IncuCyte® ClearView plate for invasion assays.

- Data generated with nuclear red labelled HT1080 cells (1K/well) grown directly on the ClearVeiw plate (**left panel**) or within a layer of basement membrane extract (BME, 5mg/ml, **right panel**) and moving towards 10% FCS.
- Note larger response to FCS over 70 h for directional migration (**left panel**). In contrast, invasion through BME (**right panel**) displays a smaller response. IncuCyte® high-definition images reveal mesenchymal-like morphology and filopodia-like projections into the 3D biomatrix.
- Invasion but not migration of cells can be inhibited by the matrix metalloproteinase inhibitor, GM6001 in a concentration dependent manner.

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