**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.

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**Reproducible 96-well Assays Suitable for Screening**

- 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 chemotactic 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 (EC50 value range 19 to 33 nM) within and between days.

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**Measure Pro-Anti-Migration Effects**

- Primary T cell CKCR4 pharmacology.
  - Isolated human primary T cells (5K cells/well) were seeded onto a Matrigel® (50 µg/ml) coated ClearView plate and the pharmacologically responsive measured through the endogenous CKCR4 receptor over 30 h.
  - Data (top panel) illustrates the time course and concentration dependent response to the CKCR4 agonist SDF-1α.
  - The response with SDF-1α (50 nM, bottom panel) can be inhibited with AMD3100, known CKCR4 antagonist with an IC50 of 0.3 µM.

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**Migration Across Relevant 2D Surfaces**

- 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 a 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.

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**Invasion Through 3D Biomatrix**

- Use of IncuCyte® ClearView plate for invasion assays.
  - Data generated with nuclear red labelled HT1080 cells (15k/well) grown directly on the ClearView 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 non-migration of cells can be inhibited by the matrix metalloproteinase inhibitor, GM6001, in a concentration dependent manner.