Continuous live-cell proliferation, clustering and viability assays for T-cells, PBMCs, monocytes and B-cells

H. Campwala, N. Bevan, C. Szybul, T. Dale & D. Trezise
Essen BioScience Ltd., Welwyn Garden City, AL7 3AX UK

Overview

- Standard techniques for monitoring non-adherent immune cell physiology include flow cytometry, "³H" thymidine and ATP assays.
- These approaches are perturbing to cells and do not provide additional biological insight.
- Conventional microscopy overcomes these limitations but is infrequently used as non-adherent cells can be hard to image.
- Here, we have developed and validated continuous live-cell assays for non-adherent cells using IncuCyte® ZOOM.
- The approach is amenable to all non-adherent cells and does not interfere with their inherent biology.
- The data presented here demonstrates how simple methodology can be integrated with IncuCyte ZOOM to provide a powerful technological tool for immunology researchers.

T-cell proliferation & clustering is seeding density-dependent

- T-cells demonstrate little or no proliferation under basal conditions but rapidly proliferate when activated (e.g. by IL-2, anti-CD3, anti-CD28).
- Following activation, T-cells also form cell clusters; imaging enables quantification of this phenotype.

Automated 96-well continuous analysis

- The confluence of unstimulated PBMCs can be seen to drop over time due to the possible presence of phagocytes.

T-cell activation is stimulus and concentration-dependent

- Data shown are for PBMCs treated with combinations of IL-2, anti-CD3, and anti-CD28.

L-kynurenine inhibits T-cell proliferation and clustering

- L-kynurenine (L-kyn) is a metabolite formed from the catabolism of L-tryptophan by the enzymes IDO and TDO.
- Some cancers increase L-kyn production in a bid to block antigen-driven T-cell proliferation and induce T-cell death, thus allowing cancer cells to escape immune surveillance.
- Inhibitors of IDO and/or TDO are therefore promising therapeutic targets for the treatment of cancer.

Plate-coatings enable cells to remain in the field-of-view

- Poly-L-ornithine performs better than other coatings in supporting proliferation and providing a uniform cell distribution for imaging. Fibronectin is also suitable for most cell types but is known to induce cell proliferation.

Confluence is a validated measure of cell number

- Non-adherent cell proliferation is quantifiable with IncuCyte ZOOM and fully-validated against direct cell counting and ATP measurement.
- WI2-NS cells counted using a Scepter® and seeded at various densities onto PLO-coated 96-well plates.
- Cell number quantified using phase contrast imaging (IncuCyte ZOOM) or ATP luminescence assay.

Phase-contrast can be duplexed with cell health reagents

- Phase-contrast analysis can be duplexed with cell health reagents (e.g. IncuCyte Cytotox Reagents) and/or apoptosis markers (IncuCyte Caspase-3/7 or Annexin V Reagents).
- Shown here is data generated with Jurkat cells treated with the topoisomerase inhibitor, camptothecin.
- Concentration-response curves were generated from time-course profiles to enable determination of IC50 values.

www.essenbioscience.com