

High Content Screening in Oncology Research using an Acumen[®] eX3

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Abstract

Oncology research has been quick to adopt High Content Analysis (HCA) because of the direct relevancy of a number of applications. Traditional methods include flow cytometry and microscope-based imaging systems, however laser-scanning microplate cytometry has many advantages over these.

The Acumen eX3 microplate cytometer (TTP LabTech) offers unparalleled throughput for cell cycle screening over flow cytometry methods. Standard protocols can read an entire 384-well microplate in under 10 minutes, and this includes multiplexing the assay with e.g. mitotic index determination. Acumen eX3 can analyze adherent cells *in situ* which preserves morphological changes which may occur during treatment, unlike flow cytometry which requires cell suspensions for processing. The use of cell-permeant DNA stains has enabled multiplexing of cell cycle analysis with other biomarkers in live cells.

Acumen eX3 provides an HCA approach to identifying kinase modulators using either phospho-specific antibodies which recognize only the active form or anti-protein antibodies to determine kinase translocation from the cytoplasm into the nucleus (indicating activation). Alternatively, GFP-tagged proteins can be monitored to provide simplified and more easily automated protocols for screening.

Cell colony enumeration traditionally involves laborious and subjective counting by hand using a microscope. Microscope objectives are unable to fully visualise very large single colonies (>1mm), making analysis with CCD imagers impractical as these require multiple image capture and image stitching prior to data analysis. Acumen eX3 can visualise whole wells, identifying colonies of any size by applying a volume algorithm.

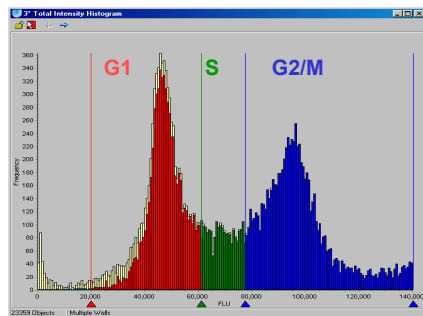
Summary

- Fast plate read times (4 – 10 minutes)
- Scans 96, 384 and 1536 plates in same time
- Small file sizes; down to Kb in screening mode
- Multiplexing – up to 4 colours per laser (12 in total)
- Rapid whole well analysis
- Robust screening data (Z')

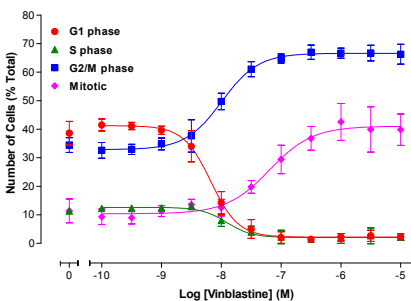
1 Multiplex Measurement of Cell Cycle Analysis and Mitotic Index

Key Features:

- Read time: 10 minutes per plate (96, 384 or 1536).
- Can be run on adherent or non-adherent cells.
- Compatible for multiplexing with accessory proteins or morphological readouts.
- Supports FCS file export.



Multiplexed cell cycle and mitotic index analysis on Vinblastine treated cells



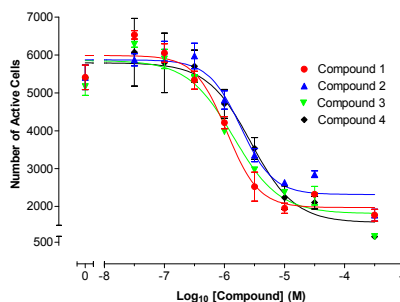
Fixed cells stained with propidium iodide (cell cycle analysis) and anti-phosphorylated Histone H3 (FITC 2° conjugate; mitotic index).

4 Protein Translocation for the Analysis of Protein Kinase Signalling

Key Features:

- Reports data for every cell within the well, generating statistically robust data: overcomes problems of variable stimulation and random cell distribution.
- Permits use of low cell numbers (1-2,000 per well) decreasing the demands on cell culture departments.
- Normalisation of biological responses to every cell in the well; offers a simple toxicity or proliferation readout with every test.

Analysis of NFκB Translocation Using an Acumen eX3



Comparison of NFκB translocation data on an Acumen eX3 and Cellomics ArrayScan

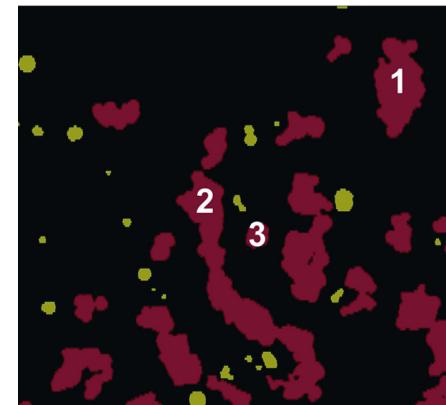
Compound	Cellomics ArrayScan IC ₅₀ (nM)	Acumen eX3 IC ₅₀ (nM)
1	800	1090
2	1460	1870
3	980	1410
4	3020	2770

Acumen eX3 and Cellomics ArrayScan platforms return comparable data and IC₅₀ values for NFκB translocation assays.

6 Cell Colony Formation

Key Features:

- Whole well scanning: Field of view not limiting. All colonies in the well, regardless of size, are accurately determined.
- Can reliably determine number of cells required to distinguish between a cluster or colony.
- Volume algorithm accounts for different shaped colonies.
- Acumen eX3 provides a significant increase in throughput, suitable for primary screens.



Colony Number	Area (μm ²)	Volume (μm ³)	Volume:Area Ratio
1	14250	1718000	121
2	30520	29870000	979
3	1870	62020	33