Cell Cycle Analysis using an Acumen® eX3 Microplate Cytometer

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Abstract

The cell cycle represents one of the most fundamental and important processes in eukaryotic cells, culminating in cell growth and division into two daughter cells. Defects in cell cycle regulation are a characteristic feature of tumour cells and mutations in the genes involved in controlling the cell cycle are extremely common in cancer. Monitoring dysfunctional cell cycle regulation is thus the focus of intense interest, since it provides an opportunity to discover new targets for anti-cancer drugs and improved therapeutics.

Traditionally, cell cycle analysis has been performed using flow cytometry which measure changes in DNA content following staining with fluorescent dye. The main disadvantages of this technique are low throughput, use of large number of cells and the inability to analyse adherent cell lines *in situ*. To address such issues, we have developed a cell cycle analysis method using an Acumen® eX3 fluorescence microplate cytometer, capable of reading an entire 384 well microplate in under 10 minutes. The method can perform such analyses on cells *in situ*, markedly simplifying sample preparation and automation.

The Acumen eX3 offers up to three laser excitation lines at 405nm, 488nm and 633nm. Such capability enables cell cycle analysis using a broad range of DNA stains including traditional ones such as DAPI, Hoechst and propidium iodide. Cell cycle analysis is typically performed on permeabilised or fixed cells using a cell-impermeant nucleic acid stain, but is also possible using live cells and a cell-permeant nucleic acid stain. While the choices for fixed cell staining are varied, there are only a few examples of useful cell-permeant nucleic acid stains, including the Vybrant® DyeCycle™ reagents (1).

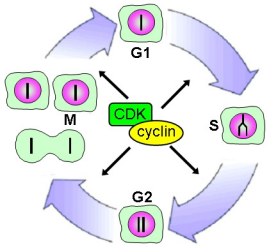
In this study, we demonstrate the use of 405nm and 488nm laser excitation for cell cycle analysis using an Acumen eX3. Data from RNAi library screening and multiplexed protocols are presented to demonstrate the flexibility of the screening platform got high content screening.

Conclusion

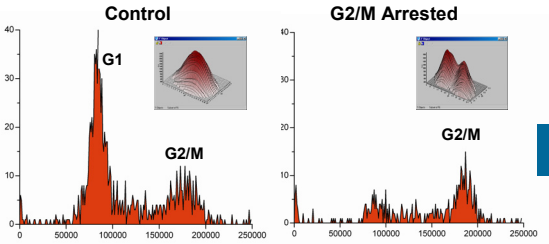
- The Acumen eX3 microplate cytometer offers rapid *in situ* cell cycle analysis – 50 times faster than flow cytometry
- A multiple laser Acumen eX3 is compatible with a broad range of DNA stains applicable for cell cycle analysis
- Protocols allow multiplexing of cell cycle analysis with other biomarkers such as those for mitotic index
- DNA histograms exported from an Acumen eX3 microplate cytometer can be analysed by the ModFit LT analysis software

1. Huth U, et al., (2004) Cytometry A. 57, 10-21.

1 Schematic of the Cell Cycle

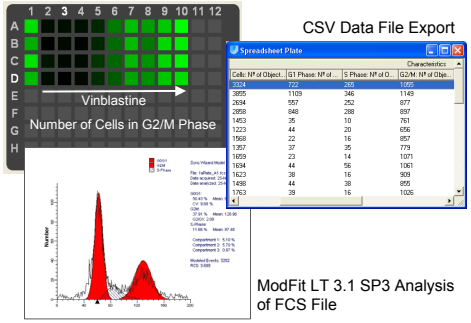


2 DNA Histograms of HeLa Cells Labelled with Propidium Iodide



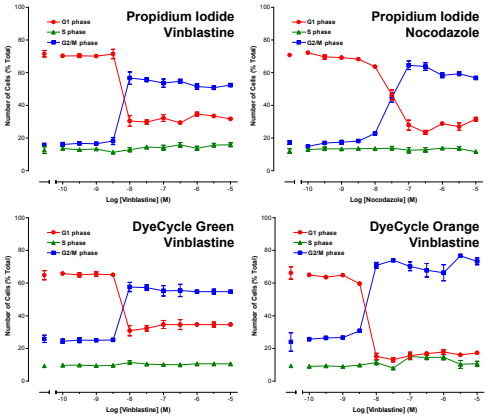
HeLa cells were fixed *in situ* using ethanol, treated with RNase (0.2 mg/mL) and stained with propidium iodide (10 μM). Analysis was performed on an Acumen eX3 microplate cytometer using 488nm laser excitation. Insets: 3D fluorescence intensity plots of single nuclei.

3 Analysis and Reporting of Cell Cycle Experiments in Explorer Software

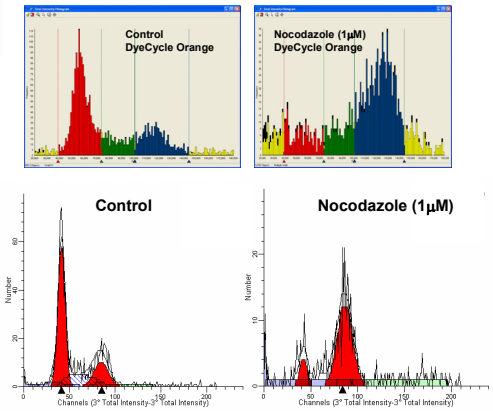


ModFit LT 3.1 SP3 Analysis of FCS File

4 Cell Cycle Analysis: Comparison of DNA Stains

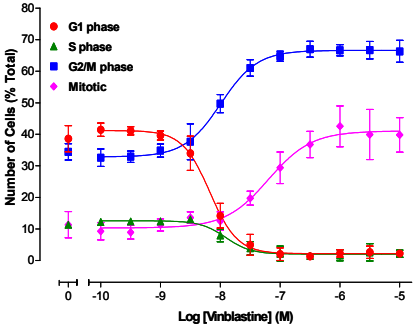


5 Cell Cycle Analysis in HeLa Cells using Microplate Cytometry



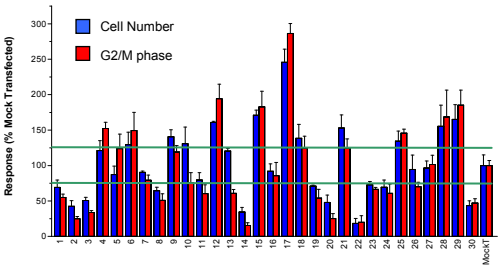
Day-old HeLa cells (2,000 per well) were treated with nocodazole for 22 hours. Cultures were labelled *in situ* with Vybrant® DyeCycle™ Orange (5 μM for 30 min at RT). Analysis was performed on an Acumen eX3 microplate cytometer using 488 nm excitation. Data was exported in FCS 3.0 format and analyzed using ModFit 3.1 LT SP3 (Verity House Software). Multiplex protocols are available for simultaneous mitotic index (anti-pH3) and cell cycle determination.

6 Multiplexed Cell Cycle Analysis and Mitotic Index Determination



Multiplex measurement of cell cycle and mitotic index analysis in CHO cells. After incubation with vinblastine for 22 hours, cells were fixed and stained with anti-phosphorylated Histone H3 (FITC secondary conjugate) to determine mitotic index and propidium iodide for cell cycle analysis. Simultaneous two colour analysis was performed on an Acumen eX3 using 488nm laser excitation. Mitotic cells were classified using Green (FITC) Peak Intensity. All data were normalised to the total number of cells in each well to compensate for variation in seeding density and perturbation of cell proliferation.

7 RNAi Library Profiling



Pre-miR™ miRNA molecules (Ambion), which act as synthetic miRNAs, were transfected into BJ cells. Cell proliferation and cell cycle data were obtained in a single scan of PI stained nuclei. Cells were sorted into G1, S and G2/M populations according to total fluorescence intensity. A high correlation between the level of cell growth and the number of cells undergoing cell division (G2/M phases) was observed, supporting the use of the multiplex assay for identifying miRNA inhibitors able to block one or more stages of the cell cycle.

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