Cell Cycle Analysis using Microplate Cytometry: A Comparison of Laser and Dye Combinations

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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 Explorer® 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.

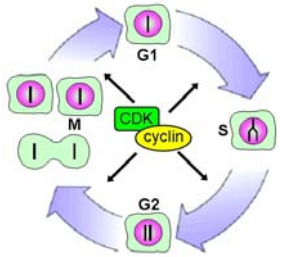
Cell cycle analysis is typically performed on permeabilised or fixed cells using a cell-impermeant nucleic acid stain, but is also possible using live cells and a cell-permeant nucleic acid stain. For fixed cell protocols the most commonly used DNA dye is propidium iodide. It has the advantage of being excited by 488 nm light and can be used on both flow and microplate cytometers. 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 compared the performance of Vybrant® DyeCycle™ Violet stain and Vybrant® DyeCycle™ Green stain, excited by 405 nm and 488 nm laser lines respectively, with propidium iodide on both flow and microplate cytometers. The results demonstrated a high degree of correlation between the different DNA stains and cytometers, supporting their integration in automated cell cycle protocols.

Conclusion

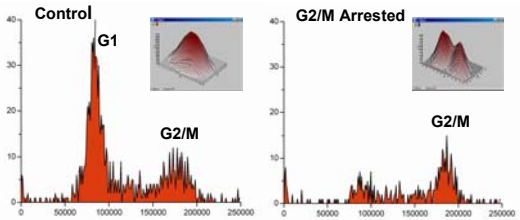
- Microplate cytometry offers rapid *in situ* cell cycle analysis of adherent cells.
- Vybrant® DyeCycle™ DNA stains offers comparable cell cycle analysis to propidium iodide without cell fixation or RNase treatment.
- Vybrant® DyeCycle™ DNA stains allow multiplexing of cell cycle analysis with other biomarkers in live cells.
- DNA histograms exported from an Acumen Explorer 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

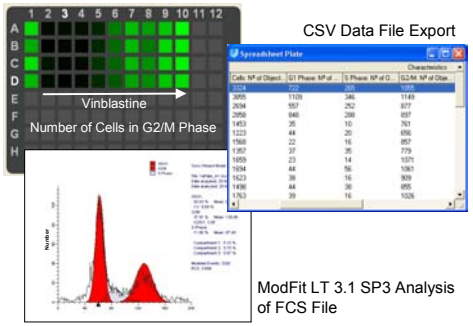


2 DNA Histograms of HeLa Cells Labelled with Propidium Iodide

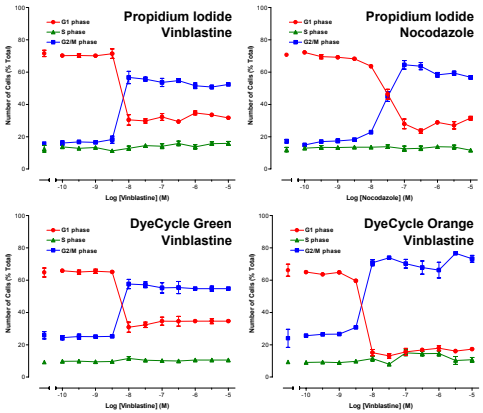


HeLa cells were fixed *in situ* using ethanol, treated with RNase (0.2mg / mL) and stained with propidium iodide (10 μM). Analysis was performed on an Acumen Explorer using 488nm laser excitation. Insets: 3D fluorescence intensity plots of single nuclei.

3 Analysis and Reporting of Cell Cycle Experiments in Explorer Software



4 Cell Cycle Analysis in HeLa Cells using Microplate Cytometry



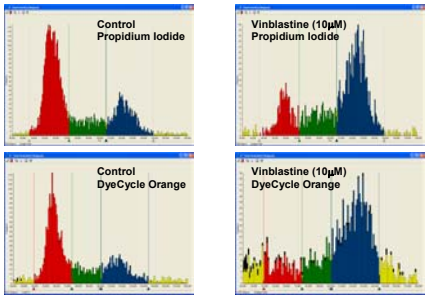
5 Correlation of Vinblastine Activity in CHO and HeLa Cells

Day-old CHO or HeLa cells (2,000 per well) were treated with vinblastine for 22 hours. Cultures were labelled *in situ* with Vybrant® DyeCycle™ Orange (5 μM for 30 min at RT) or propidium iodide (as Fig 2). Analysis was performed on an Acumen Explorer using 405nm or 488nm excitation. Data are derived from at least 3 independent experiments.

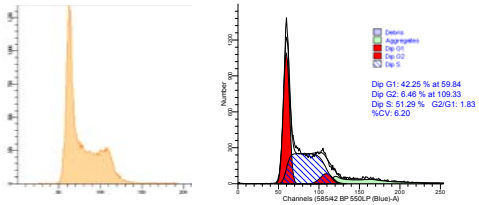
CHO	G1 (pEC50)	G2/M (pEC50)
Propidium Iodide	8.19	8.04
DyeCycle™ Violet	8.15	8.00
DyeCycle™ Green	7.58	7.62

HeLa	G1 (pEC50)	G2/M (pEC50)
Propidium Iodide	8.32	8.40
DyeCycle™ Green	8.38	8.36
DyeCycle™ Orange	8.38	8.37

6 DNA Histograms in HeLa Cells Obtained using Microplate Cytometry

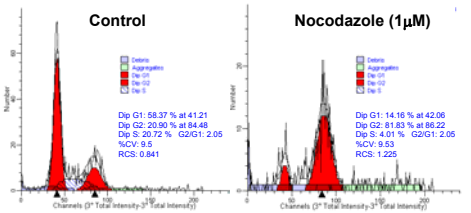


7 Analysis of Vybrant® DyeCycle™ Orange using Flow Cytometry



CHO were suspended in HBSS at 1 x10^6 cells/mL and labelled with Vybrant® DyeCycle™ Orange (5 μM for 30 min at RT). Analysis was performed on a BD LSRII flow cytometer using 488 nm excitation and fluorescence collected using a 585/42 bandpass filter. The histogram was gated on live cells only.

8 Analysis of Vybrant® DyeCycle™ Orange 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 Explorer 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).

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