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

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Abstract

Many anticancer agents and carcinogens are DNA damaging chemicals and exposure to such chemicals results in the deregulation of cell cycle progression. The cell cycle is thus a target in oncology research, making the ability to monitor the effects of such agents on the cell cycle an important part of the drug development process. Traditionally, cell cycle analysis has been performed using flow cytometry.

For improved screening capability, we have developed a cell cycle analysis method using an Acumen Explorer fluorescence microplate cytometer, capable of reading an entire 384 well microplate in about 10 minutes. The method can perform such analyses on fixed cells *in situ*, markedly simplifying sample preparation. Here, we compare the performance of different combinations of laser excitation and fluorescent DNA labelling dyes on an Acumen Explorer microplate cytometer.

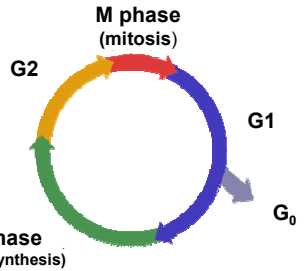
Introduction

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 (1,2). 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 fixed 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 (3,4). 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. When using a 405 nm laser line, Molecular Probes offers Vybrant® DyeCycle™ Violet stain (5). Here, we compare the performance of combinations of laser excitation and fluorescent DNA labelling dyes on Acumen Explorer microplate cytometers equipped with either 405 nm or 488 nm laser lines.

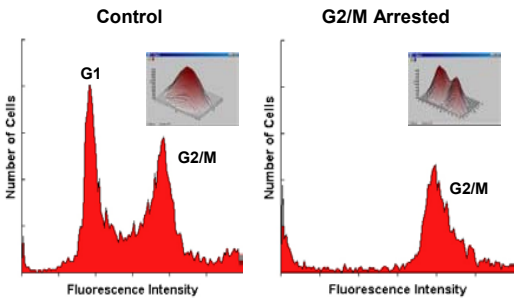
1 Schematic of the Cell Cycle



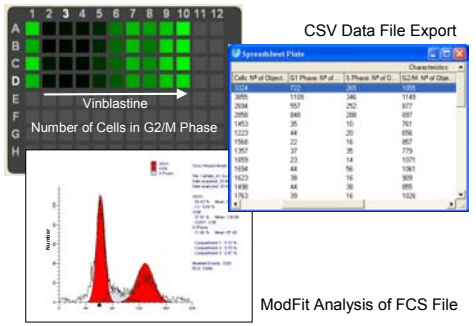
2 Assay Protocol

- CHO cells (2,000 cells per well) were treated with vinblastine for 22 hours @ 37°C / 5% CO_2.
- For studies on propidium iodide, cells were fixed with cold ethanol (100%, -20°C), washed with PBS and incubated with RNase in PBS (0.2 mg/mL, DNase free) for 4 hours at room temperature.
- Subsequently, nuclei were stained with one of the following for 30 min at room temperature; propidium iodide (3 µM; Sigma) or Vybrant® DyeCycle™ Violet stain (5 µM; Invitrogen).
- The plate was scanned on an Acumen Explorer fluorescence microplate cytometer using either 405 nm or 488 nm laser excitation.

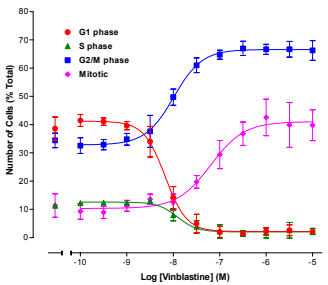
3 DNA Histograms of Cells Labelled with Propidium Iodide



4 Analysis and Reporting of Cell Cycle Experiments in Explorer Software

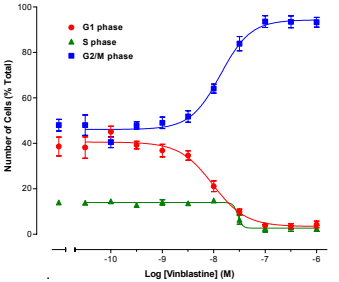


5 Multiplex Measurement of Cell Cycle Analysis and Mitotic Index



Fixed cells were stained with propidium iodide (cell cycle analysis) and anti-phosphorylated Histone H3 (FITC 2° conjugate; mitoitc index) and both analysed simultaneously on an Acumen Explorer using 488nm laser excitation.

6 Cell Cycle Analysis Using Vybrant® DyeCycle™



Live cells were stained with Vybrant® DyeCycle™ and analysed on an Acumen Explorer using 405nm laser excitation.

7 Summary of Vinblastine Results From Laser/Dye Combinations

DNA Stain	G1 Phase (pEC50)	G2/M Phase (pEC50)	n
Propidium Iodide	8.19 ± 0.06	8.04 ± 0.06	5
Vybrant® DyeCycle™	8.15 ± 0.07	8.00 ± 0.05	3

Data for propidium iodide was obtained on fixed cells following their treatment with RNase. Vybrant® DyeCycle™ DNA stain was applied directly to live cells in a more simplified assay protocol. Data (pEC50) for S Phase was highly dependent on the filters used to define G1 and G2/M phases, and therefore has been omitted from this summary.

Conclusions

- Microplate cytometry offers rapid *in situ* cell cycle analysis of adherent cells.
- Cells can be classified as G1, S or G2/M phase by their total fluorescence intensity.
- Vybrant® DyeCycle™ DNA stain offers comparable cell cycle analysis to propidium iodide without the requirement for RNase treatment.
- Vybrant® DyeCycle™ DNA stain allows simultaneous co-staining of live cells for other parameters.

References

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