Use of Far-Red Emitting DNA Dye DRAQ5 for Cell Cycle Analysis with Microplate Cytometry

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Abstract

There is an increasing demand for multiplexing in high content assays to maximise data generation and allow correlation across multiple readouts.

Currently, the degree of multiplexing can be limited by the available reagents, with the majority of fluorescent probes optimised for excitation at 488 nm. The use of fluorescent probes with spectral profiles that overlap hinders their use in multiplexing assays. Another limitation is that many fluorescence detection systems excite and detect over a narrow wavelength range, making them incompatible with certain probes.

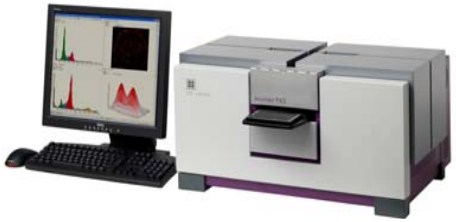
Laser-scanning fluorescence microplate cytometers, such as the Acumen® eX3 (TTP LabTech Ltd, Melbourn, UK), offer 405nm, 488nm and 633nm laser excitation in a single instrument. This technology is heavily used in oncology research including cell proliferation and cell cycle analysis using the DNA stains propidium iodide (488nm excitation) and Hoechst 34580 (405nm). Here, we describe the use of DRAQ5™ (633nm) on an Acumen eX3.

Use of DRAQ5 has become popular since it is a far-red fluorescent DNA dye that can be used in live and fixed cells in combination with other common fluorophores, especially GFP fusions and FITC-tags without spectral emission overlap. Thus DRAQ5 offers great potential for multiplexing DNA content analysis with immunodetection assays.

Conclusion

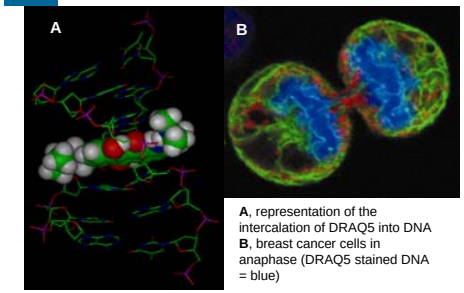
- Cell cycle analysis can be assessed in cells with 405, 488 and 633 nm excitable DNA stains using an Acumen eX3
- DRAQ5 provides quantitative cell cycle analysis comparable to propidium iodide and Hoechst 34580
- Its far-red emission makes DRAQ5 ideal for multiplexing with other common fluorophores, especially GFP fusions and FITC-tags
- Screening performance of microplate cytometry and the spectral properties of DRAQ5 make this a strong combination in HCS.

1 Acumen eX3 Microplate Cytometer



The Acumen eX3 laser scanning fluorescence microplate cytometer offers triple laser excitation in a compact bench top unit. This design enables a wide range of high content assays to be performed at high throughput, especially when the instrument is fully integrated. Patented signal thresholding methods enable 'on-the-fly' cytometric analysis and dramatically reduce file sizes to around 50Kb in HTS screening mode.

2 DRAQ5 Features and Benefits



A, representation of the intercalation of DRAQ5 into DNA
B, breast cancer cells in anaphase (DRAQ5 stained DNA = blue)

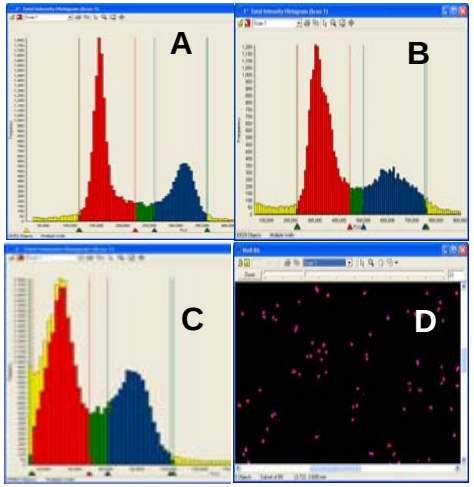
- Visible excitation-can be used on a wide range of instrument platforms
- Far-red emission-no overlap with GFP/FITC therefore no need to compensate
- No appreciable auto-fluorescence - no need to wash out
- DNA specific, stoichiometric-cell cycle analysis
- Live cells and fixed cells

3 Table of Common Excitable Fluorescent Reagents

405 nm	488 nm	633 nm
Hoechst	Propidium Iodide	DRAQ5
DyeCycle™ Violet	DyeCycle™ Orange	TO-PRO-3
Alexa 405	Calcein-AM	VITA Blue
Quantum Dots	Alexa 488	Alexa 633
FuraRedHI	FITC	Allophycocyanin
Pacific Blue	Phycoerythrin	Cy5
AmCyan	eGFP	HcRed1

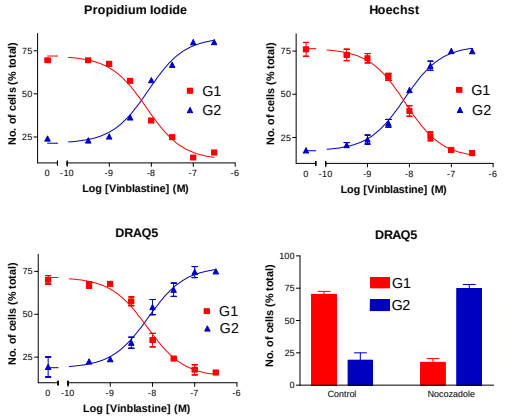
Acumen eX3's multi-laser excitation and ability to acquire up to 12 channels of fluorescent data per scan enables use of a broad range of fluorescent dyes, probes and proteins for enhanced multiplexing within assays. Since nuclear staining is not required to locate the cells, all probes may be used for reporting biological responses. By offering a comparable range of dyes to that of white light source instrumentation, an Acumen eX3 simplifies transfer of assays from microscope-based CCD Imagers onto the instrument for primary screening.

4 DNA Histograms: Comparison of DNA Stains Excited at 405, 488 or 633 nm



HeLa cells (2,000 per well) were labelled *in situ* with, A, propidium iodide (10 μM); B, Hoechst 34580 (10 μM); C, DRAQ5 (5 μM); D, Well view image of DRAQ5 treated cells in G2/M block (vinblastine; 0.1 μM). Analysis was performed on an Acumen eX3 microplate cytometer using 405, 488 or 633 nm excitation.

5 Cell Cycle Analysis: Comparison of DRAQ5 versus other DNA Stains



HeLa cells (2,000 per well) were treated with vinblastine for 22 hours @ 37°C / 5% CO2. Cells were fixed with cold ethanol (80%, -20°C), washed with PBS. For propidium iodide staining, cells were incubated with RNase in PBS (0.2 mg/mL, DNase free) for 1 hour at 37°C.The cells were labelled with propidium iodide (10 μM); Hoechst 34580 (10 μM); Draq5 (5 μM). Analysis was performed on an Acumen eX3 microplate cytometer using 405, 488 or 633 nm excitation. Cell cycle analysis data using DRAQ5 is comparable to that obtained with propidium iodide and Hoechst.

6 Throughput and Data Storage for Acumen eX3

	96	384	1536
Plate Read Time (whole well)	9.15	10.24	10.26
Plate Read Time (HTS)	4.13	4.8	6.67
Plates per 24h	350	300	216
Wells per 24h	34,000	115,000	330,000
Total Data for 24h operation	17.5 Mb	60 Mb	170 Mb

An Acumen eX3 scans on an area and not well basis, thus scan times are virtually identical for any SBS format microplate. Typically plate cycle times of 10 minutes are achievable but these can be further cut by scanning reduced well areas. Several hundred plates can be scanned per day with minimal requirements for data storage. Data for scanning resolution of 1 μm x 8 μm using a single laser Acumen eX3. Plate read times given in minutes.

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