

Utility of 405nm-excitable dyes in High Content Screening using an Acumen Explorer Microplate Cytometer

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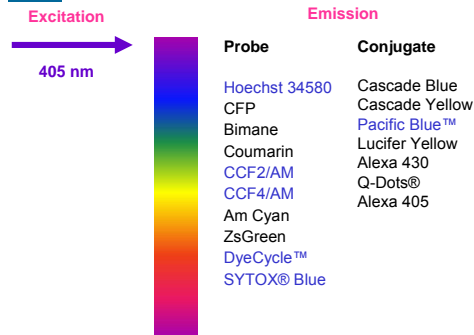
Introduction

Shorter-wavelength amine-reactive fluorophores are infrequently used for preparing bioconjugates because dyes excited with longer wavelengths, and therefore lower energy, are widely available and less likely to cause photodamage to labeled biomolecules. Moreover, many cells and tissues autofluoresce when excited with ultraviolet (UV) light and thus preclude the use of blue-fluorescent conjugates in a number of applications. However, for certain multicolour fluorescence applications, including immunofluorescence, a blue-fluorescent probe provides a contrasting colour that is easily distinguished from the green, yellow, orange or red fluorescence of the longer-wavelength probes.

The Acumen Explorer is a fluorescent microplate cytometer equipped with either a 405nm or 488 nm laser widely used in HCS. The instrument offers rapid read and analysis times of 96-1536 well plates (typically 5-10 minutes) compatible with the sustained use of high content methods in primary screens. In addition, small file sizes (>50kB in screening mode) are produced, therefore removing the requirement for expensive data storage solutions in compound screening programs. These features have made the Acumen Explorer a widely used High Content Screening platform.

In this study, we have used an Acumen Explorer equipped with a violet 405nm laser in conjunction with a selection of shorter-wavelength amine-reactive fluorophores (Invitrogen, Molecular Probes). We have demonstrated the utility of the Acumen Explorer and the blue fluorescent probes in HCS using Pacific Blue™ Annexin V conjugates for apoptosis assays, SYTOX® Blue stain for vitality assays, DyeCycle™ stains for cell cycle analysis and β-lactamase-based assay to determine GPCR activation.

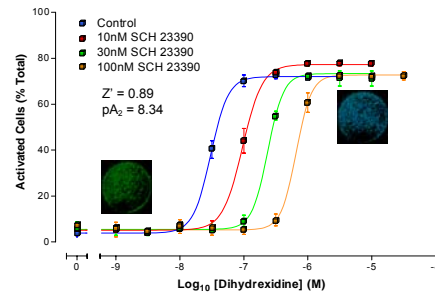
1 Fluorescent Dyes suitable for 405 nm Excitation



The availability of an Acumen Explorer equipped with a 405nm (violet) solid-state laser, creates opportunities for utilising new dyes as shown above and enhances the multiplexing capability for High Content Screening.

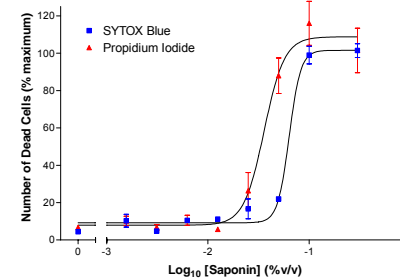
2 Detecting the FRET Response of the GeneBLazer® Technology.

Acumen Explorer equipped with 405nm laser line can simultaneously scan both the blue (β-lactamase expressing) and green spectrum (β-lactamase negative) to discriminate active from inactive cells.



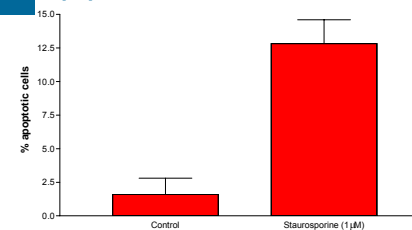
SCH-23390-concentration dependent shift of dihydropyridine curves (data represent means ± S.D. of 4 replicates and are representative of results obtained from 3 separate experiments). Insets are well views of inactive (green) and active (blue) cells.

3 Comparison of PI and SYTOX® Blue in Analysing Cell Death



Cells were treated with saponin as shown, and stained with either Sytox Blue or Propidium iodide. The Sytox Blue was analysed on an Acumen Explorer using 405 nm laser excitation. The propidium iodide was analysed on an Acumen Explorer using 488 nm laser excitation.

4 Apoptosis

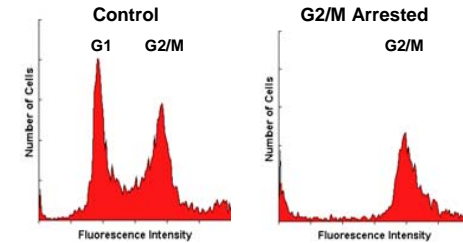


Cells were treated with staurosporine as shown, and apoptotic cells detected with Pacific Blue on an Acumen Explorer using 405 nm laser

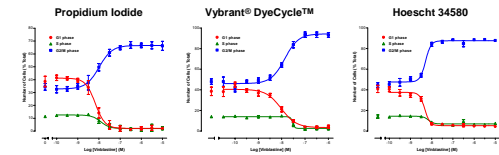
Conclusions

- High content analysis of β-lactamase reporter gene assays reports data on a per-cell basis suitable for antagonist profiling.
- Vybrant® DyeCycle™ DNA stain offers comparable cell cycle analysis to propidium iodide without the requirement for RNase treatment.
- SYTOX Blue is a comparable dye
- Short wavelength fluorophores are compatible with an Acumen Explorer equipped with a 405nm laser, enhancing multiplexing capability in high content screening.

5 DNA Histograms of Cells Labelled with Vybrant® DyeCycle™



6 Cell Cycle Analysis using an Acumen Explorer



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
Hoescht 34580	8.34 ± 0.05	8.30 ± 0.04	3

Live cells were stained with Vybrant® DyeCycle™ or Hoechst and analysed on an Acumen Explorer using 405nm laser excitation. Fixed cells were stained with Propidium Iodide and scanned on a 488 nm Acumen Explorer as a control

References

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