

Epicocconone™, a cell-permeable fluorophore for staining cytoplasmic compartments of live cells

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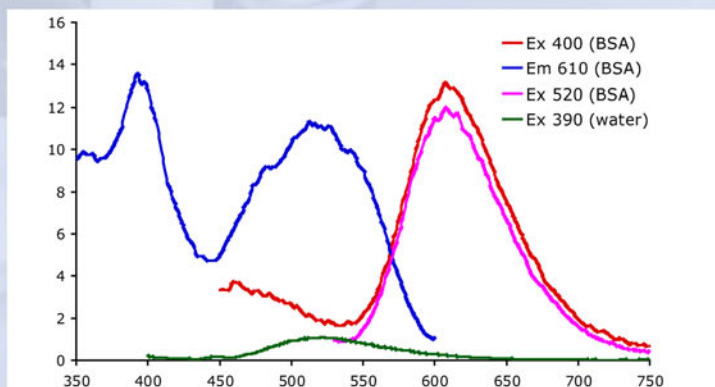
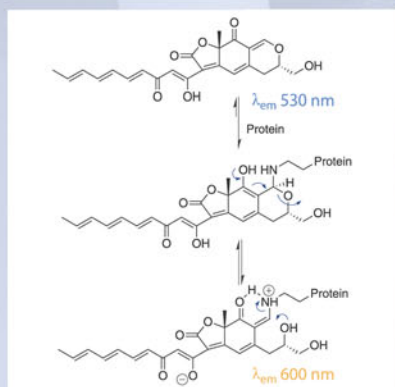
INTRODUCTION

Fluorescent labeling of sub-cellular structures can be achieved using a variety of fluorescent stains, fluorescently-labeled antibodies or co-expression of a GFP-fusion protein. Often cells must be permeabilized to allow entry of the fluorescent stain or labeled antibody or genetically altered to express GFP. It is often impossible to wash away excess label from intracellular compartments or from inside tissues, resulting in high background. Some stains are also cytotoxic limiting their use for live cell imaging. The ideal fluorescent stain would be cell permeable, non-toxic, readily distinguished from unbound stain and able to be multiplexed with other common stains (eg. by having a long Stokes' shift).

Epicocconone is a recently discovered, low molecular weight (MW 410), water-soluble, fluorescent natural product from the fungus *Epicoccum nigrum*¹ that fulfills many of these criteria and which has potential in cellular staining. This compound spontaneously, and reversibly, conjugates to lysine residues in proteins yielding an intensely fluorescent orange/red product that is easily distinguished from unconjugated stain. In hydrophobic environments, such as within cell membranes and hydrophobic pockets in proteins, the quantum yield of this fluorophore increases markedly.

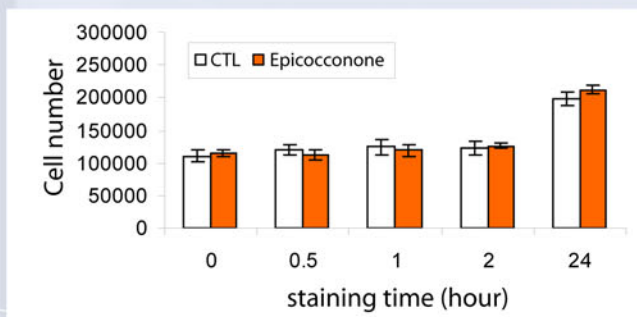
Epicocconone is excitable by common lasers such as violet GaN (400-410 nm), argon Ar ion (488 nm), frequency doubled Nd:YAG (532 nm) and He-Neon (543 nm), enabling analysis by standard fluorescence instrumentation (fluorescence and confocal microscopy, flow cytometry, etc).

EPICOCCONONE STRUCTURE & SPECTRAL CHARACTERISTICS

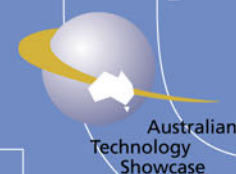


LOW CYTOTOXICITY

Epicocconone™ had no significant effect on the growth rate of a human colon cancer cell line, HCT 116 over 24 hours at a concentration of 12 μ M, which is similar or higher than typically used for live cell staining.



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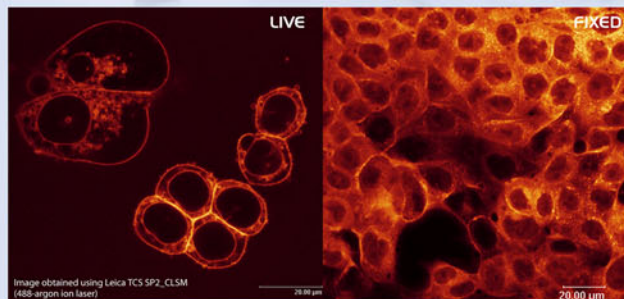
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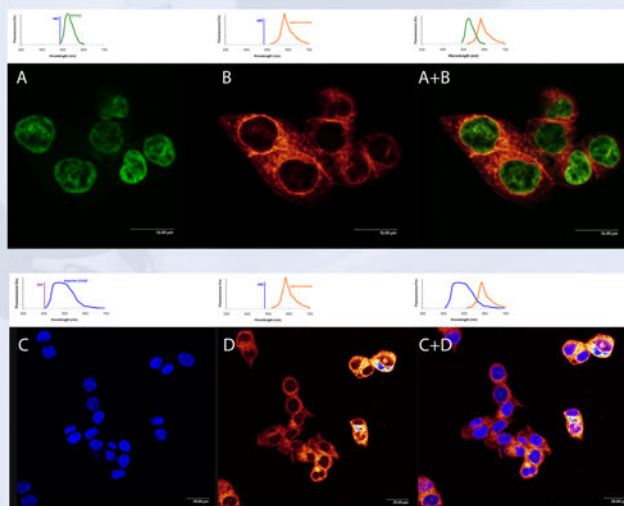
LIVE AND FIXED CELL STAINING

Epicocconone™ is a small neutral molecule that freely diffuses across plasma membranes of both viable and fixed mammalian cells without the need for permeabilizing agents that are required for antibody linked or small ionic fluorophores.



MULTIPLEXING

The long Stokes' shift of **Epicocconone™** ($\lambda_{ex} = 395$ or 495 nm; $\lambda_{em} = 584$ nm) stained cells makes this stain a useful partner for both blue emitting fluorophores (eg Hoechst 33342; $\lambda_{em} = 479$ nm) and green emitting (eg SYTOX; $\lambda_{em} = 524$ nm) fluorophores.



Cells were fixed and dual stained with SYTOX-green (nucleic acids) and **Epicocconone™**. Image was obtained using a single laser (488-argon ion) in Leica TCS SP2.

Cells were fixed and dual stained with Hoechst 33342 (DNA) and **Epicocconone™**. Image was obtained using two wavelengths (405 nm for Hoechst and 488 nm for **Epicocconone™**).

CONCLUSIONS

Epicocconone™ has a number of useful characteristics for cellular imaging including:

- The spectral characteristics (both emission maxima and quantum yield) of the fluorophore change significantly when cells are stained. This enables cells to be brightly stained against a very low fluorescence background without the need to wash away unbound fluorophore.
- Live cells are readily permeable to **Epicocconone™** and do not require any pretreatment to allow the stain to be taken up.
- **Epicocconone™** does not affect the growth rate of a wide range of cell types (bacteria, yeast, mammal) at concentrations similar to those used in staining.
- **Epicocconone™** is excited by a variety of light sources used in standard fluorescence based instrumentation.
- The long Stokes' shift of **Epicocconone™** makes it ideal for use with a wide variety of short Stokes' shift fluorophores in multiplex assays.

The technical features of **Epicocconone™** suggest it has wide utility as a fluorescent stain for cellular imaging.

REFERENCE

1. Bell, P.J.L., and Karuso, P. (2003). *J. Am. Chem. Soc.* **125**, 9304.

Epicocconone™ is available from **FLUOROTECHNICS Pty Limited**
(www.fluorotechnics.com/epic.asp)

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