FT-CL0731

LavaCell™ Protein Live Cells Stain

Product Description

Name :	**LavaCell™ Protein Live Cells Stain**
Catalog Number :	CL0731, 200µl CL0732, 1ml contains DMSO and Epicocconone dye (store at –20°C) (M)

LavaCell is a **cell-permeable** fluorophore for staining cytoplasmic compartments of live cells. It is **non-toxic** and stains cells fluorescent **orange**, but it do **not need to remove excess stain** before imaging. It can be **multiplexed** with other fluorophores such as blue-emitting Hoechst 33342 or green-emitting SX.

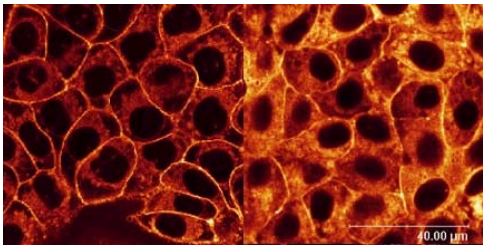
FEATURES

the dye:

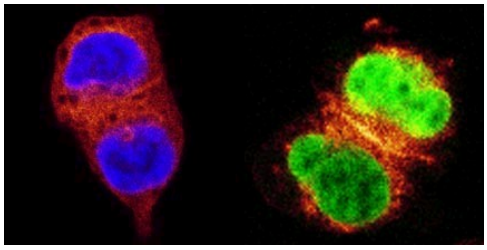
- a water soluble fluorescent stain and a small (mw 410), neutral molecule
- does not fluoresce until it interacts with proteins
- is highly fluorescent
- has a long Stokes' shift

staining cells:

- does not affect cell growth rate or viability
- readily permeates into cells
- do need to wash away unbound stain
- suitable for live cell imaging
- suitable for multiplexing



Live Cells Fixed Cells



HCT cells dual stained with Epicocconone and Hoechst™ 33342 HCT cells dual stained with

Introduction

Fluorescent labeling of sub-cellular structures can be achieved using a variety of fluorescent stains1 [2], fluorescently-labeled antibodies [3] or co-expression of a GFP-fusion protein [4, 5]. Generally cells must be permeabilized to allow entry of the fluorescent stain or labeled antibody or genetically altered to in order 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 or affect cellular processes limiting their uses for live cell imaging. The ideal fluorescent stain for intracellular imaging would be cell permeable, non-toxic, readily distinguished from unbound stain and able to be multiplexed with other common stains (e.g. by having a long Stokes' shift).

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Epicocconone™ is a recently discovered, low molecular weight (MW 410), water-soluble, fluorescent natural product from the fungus Epicoccum nigrum6 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 also increases markedly.

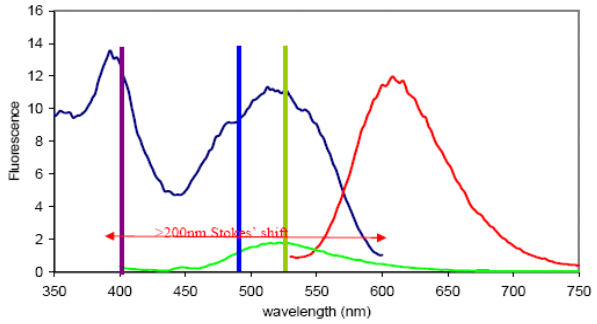

Fig.: Fluorescence spectral characteristics of epicocconone in water (green) and in the presence of Bovine Serum Albumin (BSA). Vertical lines represent common lasers used to excite the epicocconone-protein adduct (405 nm diode laser, 488 Ar ion laser and 532 frequency double Ng-YAG laser).

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).

This application note describes the use of Epicocconone™ for staining cytoplasmic structures of live mammalian cells for confocal fluorescence microscopy. The stain is non-toxic, stains cells fluorescent orange, and can be multiplexed with other fluorophores such as blue-emitting Hoechst 33342 or green-emitting SX dye.

Kit content & Storage

LavaCell kit contains 2 components

Part A comprises a natural fluorophore (epicocconone) supplied as a lyophilized powder (0.2 mg/vial).

Part B comprises DMSO and is used as a solvent for Part A.

The dye should be prepared as a 1 mg/mL solution of epicocconone in DMSO. To each vial of Part A add 200 mL of Part B. Recap the vials and vortex mix. In the larger kit (CL0732) we recommend preparing each vial of dye as required. LavaCell working solution is minimally hazardous and non-flammable; however the complete properties of the dye component have not been fully investigated.

All chemicals should be considered potentially hazardous. This product should be handled only by those persons trained in laboratory techniques, and used in accordance with the principles of good laboratory practice. Wear suitable protective clothing including laboratory overalls, safety glasses and gloves.

Stability & Storage

Upon receiving the kit, store Part A at -20°C. Part B should preferably be stored at room temperature. Part A is stable for 1 year if stored unopened at -20°C. The reconstituted solution of Part A is stable at -20 °C for 1 month.

Protocol for staining cytoplasmic compartments of live cells

Additional information can be found in the journal article : Epicocconone, a New Cell Permeable Long Stokes Shift Fluorescent Stain for Live Cell Imaging and Multiplexing - J. Fluorescence. 16: 475-82 (2006).

Materials and Methods

Reagents

1. Epicocconone in solution (2.4 mM, 1 mg epicocconone /mL dimethyl sulfoxide)

2. SX green dye solution (5 mM in DMSO)

3. Hoechst 33342 solution 8 mM in H2O (#FP-BB1340)

4. Growth medium and staining solution: Dulbecco's Modified Eagle Medium (DMEM,GIBCO, NY, USA, Catalog No.: 10569-010) containing 10 % FBS

5. Mounting medium: 50 % glycerol in 20 mM phosphate buffer (pH 8.5)

6. Phosphate buffered saline (pH 7.4 ± 0.2; Sigma-Aldrich, Sydney, Australia, Catalog No.: P-4417)

Equipment

1.. Tissue culture flask 75 cm^2 (#3275)

2. Tissue culture multi-well plate 3.5 x 1.0 cm, (#76-058-16)

3. Tissue culture multi-well plate 6.0 x 1. 5 cm (#76-037-05)

4. CultureWell™ Chambered Coverglass for cell culture (#FP-AO0861)

5. Live cell chamber & 0.16 – 42 mm cover glass (POC chamber System, #15-6108- 381)

6. Tempcontrol 37-2 digital (#0503.000)

7. TCS SP2 Confocal Laser Scanning Microscopy with DM IRE microscope (#)

8. FACSCalibur (#)

HCT 116

HCT 116 (APAF, Australia) cells, a human colon cancer cell line were maintained in tissue culture flasks containing DMEM (20 mL) at 37°C in 5 % CO2. Actively growing cells that are ~ 80 % confluent were used for cytotoxicity test and epicocconone-staining.

Cytotoxicity test

HCT 116 cells were cultured as described above and were seeded in duplicate at cell densities of approx. 2 x 10^5 cells per well in tissue culture multi-well plates (3.5 x 1.0 cm) containing 2.5 mL of DMEM growth medium. For cytotoxicity testing, the spent medium was replaced with 2 mL of fresh growth medium containing Epicocconone™ (final conc. 12 µM). Identical cultures without Epicocconone™ were used as controls. Cell growth was monitored for periods up to 24 hours by counting cell numbers using flow cytometry at 0.5, 0.5, 1, 2, and 24 hr intervals.

Staining methods

Live cells

Staining live cells with Epicocconone™ was performed in a temperature-controlled POC chamber system. HCT 116 cells were grown as a monolayer on cover glasses (42 x 0.16 mm, POC Chamber System) placed in a tissue culture multi-well plate (6.0 x 1. 5 cm) containing 2.5 mL DMEM growth medium. One cover glass with the cell monolayer was aseptically transferred and placed into the POC cell chamber, and a dye-free growth medium (~ 2 mL) was added to the chamber. The cells were observed using a DM IRE microscope. The temperature of the growth chamber was maintained at 37°C (Tempcontrol 37-2 digital) during the assay time. Immediately prior to the staining, the cells were washed twice with PBS and then incubated with 2 mL of growth medium containing Epicocconone™ (final conc. 6 –12 µM) over 30 min to obtain real time live cell images (TCS SP2).

Fixed cells

HCT 116 cells were grown as a monolayer in a CultureWell™ Chambered Coverglass (~10^5 cells/mL/well). Immediately prior to staining, the medium in the chambered coverglass was carefully decanted. The cells were washed twice with PBS by pipetting, fixed in chilled (4°C) 1 x PBS containing 4 % formaldehyde for 20 min at 4°C, and washed twice with 1 x PBS. The chambered coverglass was filled with 200 µL of DMEM growth medium containing Epicocconone™ (final conc.: 12 – 24 µM) for 30 min-staining at RT (dark). Dual staining was achieved by adding SX-green (final conc.:1.25 – 2.5 µM), or Hoechst 33342 (final conc.:1 – 4 µM).

The chambered coverglass with stained cell samples was mounted in a glycerol-based mounting medium onto a clean glass slide (Product Information for CultureWell™ coverglass, x 37000).

Confocal Laser Scanning Microscopy

Images of fluorescently stained cells were obtained using a Leica TCS SP2 confocal microscope equipped with a 405 nm diode laser (for Hoechst 33342) and a 488 nm ArKr laser (for Epicocconone™ and SX). Samples were viewed under a Leica DM IRE2 microscope (Leica Microsystems, Germany). Stained cells were imaged using a 63 x or a 100 x lens. In order to ensure uniformity for comparisons of fluorescent staining, all cell samples were analyzed the same day with the same laser scanning settings.

Image series of live cells stained with Epicocconone™ were recorded using time image series (xyt) (Leica Microsystems, user manual or http://www.univ-rouen.fr/inwerm-u413/ifrmp/tutorials/zeitbildserien_en.htm). Emission spectra of EpicocconeTM, SX and Hoechst 33342) were obtained using recording lambda series (xyλ) (Leica Microsystems, user manual or http://www.univ-rouen.fr/inserm-u413/ifrmp/tutorials/zeitbildserien_en.htm). Data from all images were processed using the Leica Confocal Software.

Results

Effect of Epicocconone™ on growth of cells

Epicocconone™ was tested for its effect on viability of a mammalian cancer cell line, HCT 116. Cell numbers of Epicocconone™-stained sample were counted by flow cytometry, and compared with those of unstained cells. 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 µM, which is similar to or higher than typically used for live cell staining (Fig. 1).

Live and fixed cells staining

Epicocconone™ was used for staining both live and fixed HCT 116. Epicocconone™, a small neutral molecule, freely diffused across the plasma membrane, and stained both live and fixed mammalian cells fluorescent orange (fig.2) without the need for permeabilizing agents that are required for antibody linked or small ionic fluorophores.

Epicocconone™ (6 – 12 µM) stained the plasma membrane of live cells fluorescent orange almost immediately (less than 1 min). This stain appears to readily permeate the plasma membrane as cytoplasmic compartments were typically stained within 30 min (http://www.fluorotechnics.com/epic.asp). An animation file showing EpicoccononeTM-staining in real-time is available on inquire.

For fixed cell staining, Epicocconone™ was used at a concentration of 24 µM. Epicocconone™ stained most of the cytoplasmic compartment of fixed cells bright orange and it did not stain nucleic acid components as indicated by counter staining with Hoechst 33342 and SX (Fig. 3).

Multiplexing

Nucleic acid stains, e.g. blue-emitting Hoechst 33342 and green-emitting SX were used as multiplexing pairs with Epicocconone™.

The xyλ scan generated an emission spectrum of Epicocconone™ ranged from 530 to 680 nm, with a maximum peak at 584 nm. Maximum emission peaks recorded by the xyλ scan for both nucleic acid stains are 479 nm (408 – 670 nm) for Hoechst 33342 and 524 nm (494 – 596 nm) for SX.

The long Stokes' shift of Epicocconone™ (λex = 395 or 495 nm; λem = 584 nm) stained cells makes this stain a useful partner for both blue emitting fluorophores (e.g. Hoechst 33342; λem 479 nm) and green emitting (e.g. SX; λem = 524 nm) fluorophores as there is very little spectral overlap between Epicocconone™ and either of the other two stains.

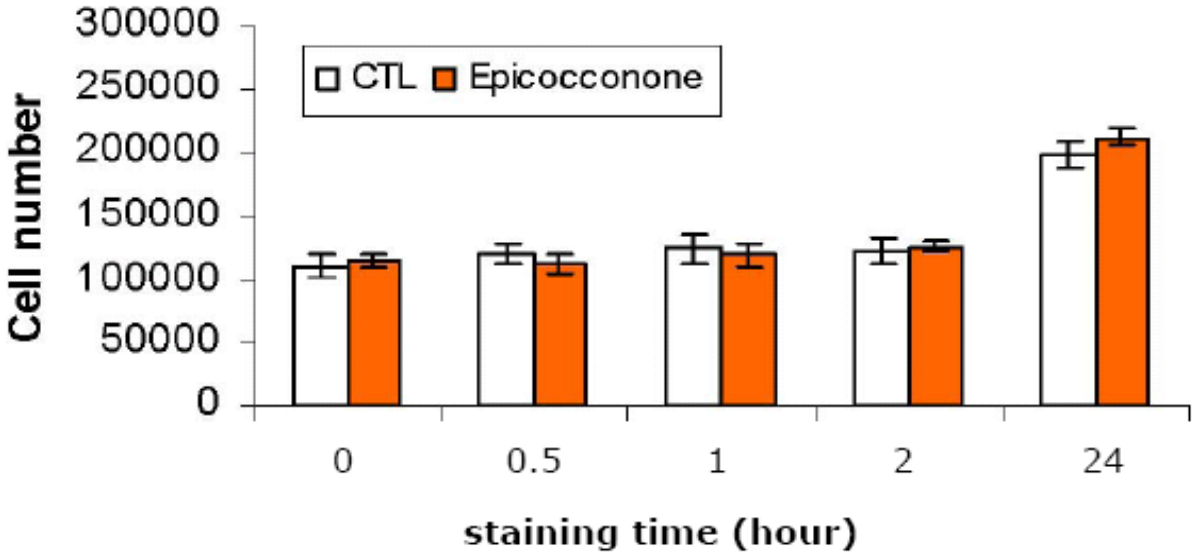

Figure 1. Low cytotoxicity of Epicocconone™ during its staining HCT 116 cells over 24 hours.

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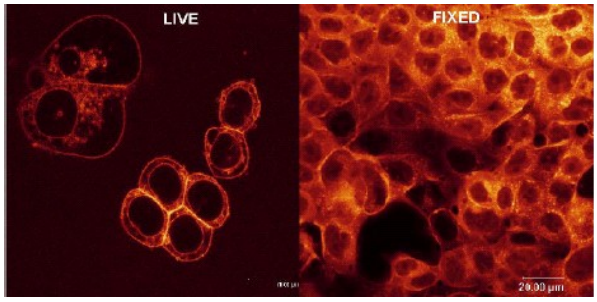

Figure 2. Confocal image showing Epicocconone™ staining both live (37°C; 30 min) and fixed cells (25°C30 min) fluorescence orange. Images were obtained using Leica Confocal equipped with (488-nm argon ion laser). Bar: 20 µm.

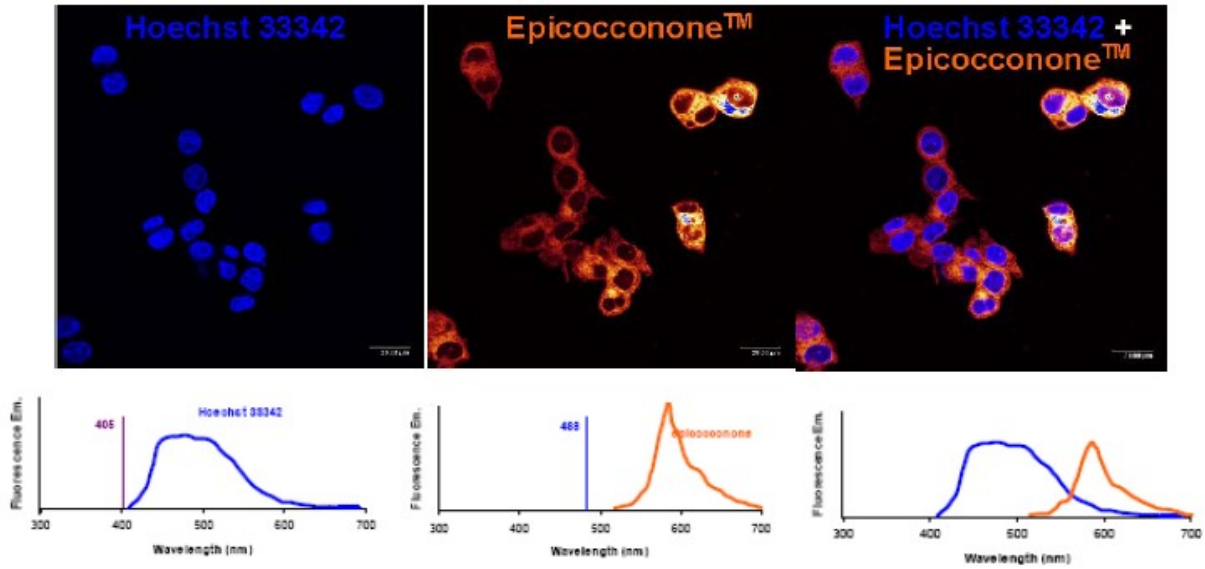

Figure 3-1. Images of HCT cells stained with Hoechst 33342 and Epicocconone™ and emission profiles. Cells were fixed and dual stained with Hoechst 33342 (DNA) and EpicoccononeTM. Images of Hoechst 33342- and EpicoccononeTM-stained cells were separately scanned by two wavelength-lasers (405 nm for Hoechst and 488 nm for EpicoccononeTM). Bar:16 µm.

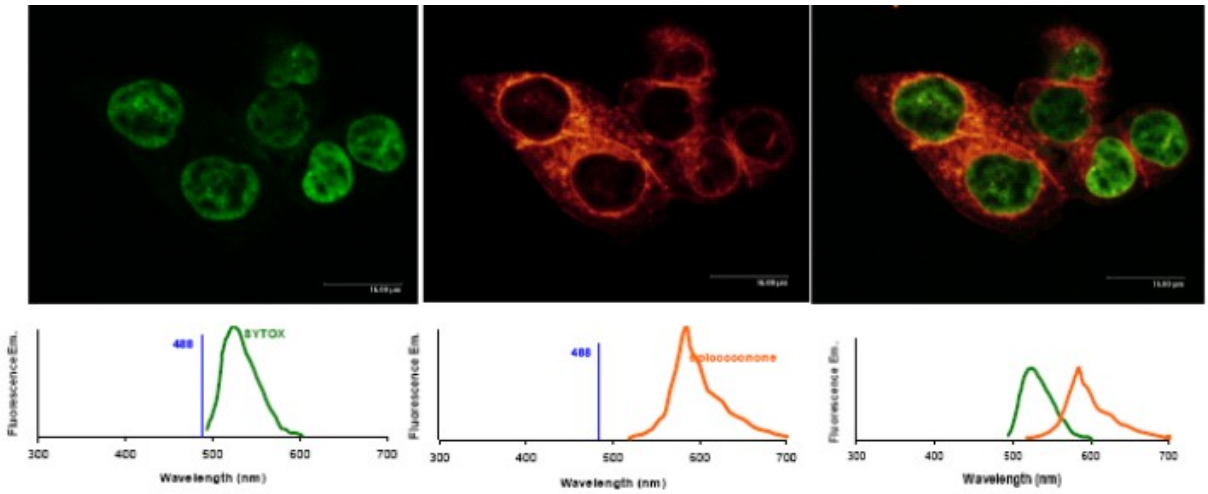

Figure 3-2. Images of HCT cells stained with SX and Epicocconone™ and emission spectrum profiles. Cells were fixed and dual stained with SX-green (nucleic acids) and EpicoccononeTM. Images were obtained using a single laser (488 nm argon laser) scanning of a cell sample in Leica Confocal TCS SP2 (channel 1 for green-emitting SX; channel 2 for orange-emitting EpicoccononeTM). Bar: 16 µm.

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Conclusions

Epicocconone™ has a number of useful characteristics for cellular imaging

1) 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.

2) Live cells are readily permeable to Epicocconone™ and do not require any pre- treatment to allow the stain to be taken up.

3) Epicocconone™ does not affect the growth rate of a wide range of cell types (bacteria, yeast, mammal; results for bacteria and yeast not shown) at concentrations similar to those used for staining.

4) Epicocconone™ is excited by a variety of light sources used in standard fluorescence based instrumentation.

5) The long Stokes' shift of Epicocconone™ makes it ideal for use with a wide variety of common short Stokes' shift fluorophores in multiplex assays.

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

References

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4 Khodjakov, A., et al. (1997) Cell Motil. Cytoskeleton 38:311

5 Nichols, B.J., et al. (2001) J. Cell Biol. 153:529

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

Legals

Related products and documents

NT-CL073n	Flow cytometric analysis of E.coli stained with LavaCell
NT-CL073o	High Throughput Anchorage-Independent Cell Colony Growth Assay with the IsoCyte-HTS Platform
#67433A	LavaPurple Gel & Blot protein Stain
#CH4191	LavaPep Peptide & Proteiin assay in solution
p.E32-E136/E146-E219	Other probes and kits for cell Biology study (incl. cell tracing, structures, apoptosis,...)

Ordering information

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