FluoProbes

FT-BS4850

EPICOCCONONE™

Product Information

Name :	**EPICOCCONONE™ protein stain**
Catalog Number :	FP-BS4850, 1 mg FP-BS4851, 200 µg
	$C_{23}H_{22}O_7$; **MW: 410**
Solubility :	DMSO, Methanol, Water

NOTE: Epicocconone is patented and available for electrophoresis gel and blot staining 67433A), peptide and protein assays (product CH4191) and cell staining (CL0730).
Please contact Interchim for any other uses (applications, manufacturing, diagnostics or therapeutics).

Figure 1. Representative fluorescence of epicocconone (left) in acetonitrile and in the presence of an amine (right)

GENERAL FEATURES

Epicocconone

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

Thus it do **not need to remove excess stain** before imaging, and
it is **ideal for multiplexing** with other fluorophores

SPECIFIC FEATURES / APPLICATIONS

gel electrophoresis and blotting

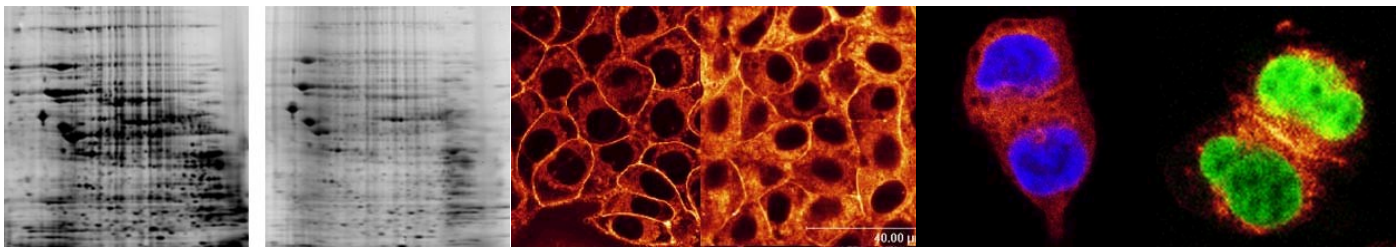
- very sensitive (5pg proteins)
- reversible staining / MS analysis, with better annotations

assaying peptides and protein in solutions

- very sensitive (5pg proteins)
- ideal for peptides dosage

cell staining

- no need to wash away unbound stain
- readily permeates into cells
- suitable for live cell imaging
- Does not affect cell growth rate or viability



TotalProteinStain : **LavaPurple** **1076 spots** Rubys stain 877 spots	Live Cells Fixed Cells	HCT cells dual stained with Epicocconone and Hoechst™ 33342 HCT cells dual stained with Epicocconone and SYTOX™

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Technical Information

Epicocconone (1), a naturally occurring biodegradable fluorescent compound extracted from a the fungus *Epicoccum nigrum*[1]. Epicocconone is a low molecular weight (410 amu), water soluble, fluorophore that spontaneously covalently binds to primary amines (such as lysine residues in proteins) to yield an intensely red-fluorescent product (figure 1). The mechanism by which the weakly green fluorescent epicocconone is converted to the highly fluorescent enamine (2) has recently been elucidated and involves the attack of nucleophilic amines[2]. This unique mechanism (figure 2) provides sensitive quantification of proteins across a wide variety platforms (e.g. in solution, gels, blots, and cells) and led directly to a number of commercial products that are becoming increasingly popular [3-6].

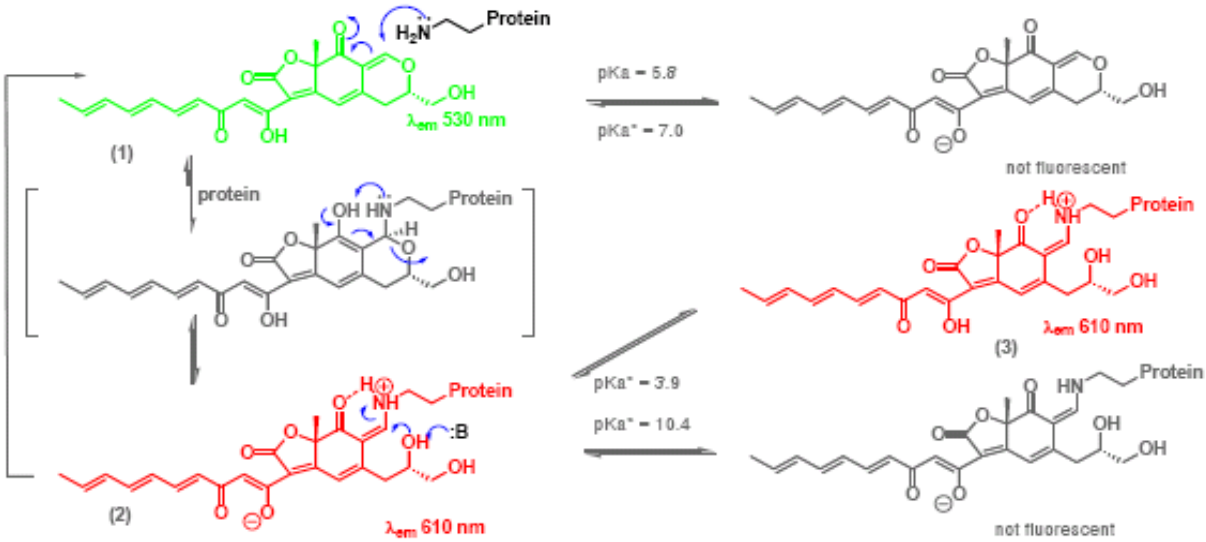

Figure 2. Spontaneous reaction of epicocconone (1) with proteins yields a fluorescent complex (2) that is readily converted back to epicocconone under neutral conditions. While the adduct 2 is quite fluorescent, a substantial increase is associated with protonation of the β-diketone side-chain (3).

Fluorescence is also pH dependant, reaching a maximum at around pH 2, which is also corresponds to the optimal stability of the epicocconone-protein complex. Thus compound 3 (Figure 2) is about 3x as fluorescent as compound 2 (Figure 3). This is the reason we store gels and blots under acidic conditions. The stability of the adduct is also quite pH dependant (Figure 4)[2]. By MALDI mass spectrometry, we have been able to determine the rate of hydrolysis of an epicocconone-peptide adduct and shown that the mechanism is base-catalysed. This has allowed us to develop the unique advantages of epicocconone in proteomics and biotechnology. That is a molecule that on covalently derivatising a protein or peptide changes spectral characteristics, allowing sensitive quantification against a non- fluorescent background but also a pH dependant removal of the fluorophore that leave the proteins or peptides free for further down-stream processing. The fluorophore can be tracelessly removed by washing a neutral pH.

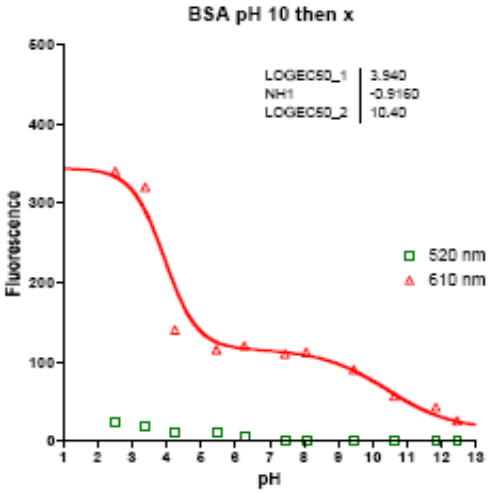

Figure 3. Fluorescence of epicocconone in the presence of protein (BSA) at varying pH. The red trace shows emission at 610 nm and the green squares are emission at 520 nm.

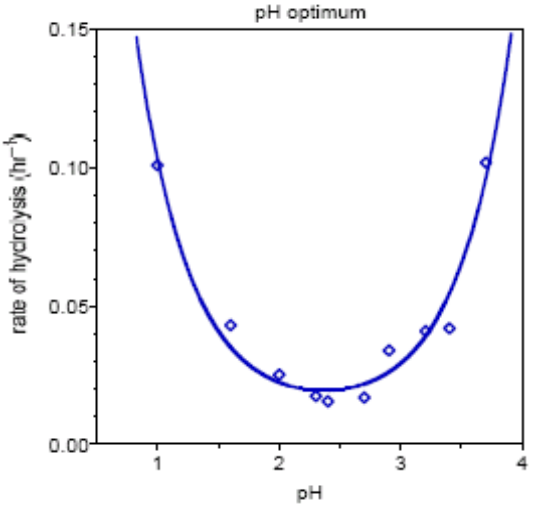

Figure 4. The optimum stability of the epicocconone adduct is achieved at about pH 2.4. Fitting the data to a general acid/base mechanism gave a pseudo-first order rate constant of kB´ = 1.03 x 105 s–1

This pH dependent, reversible binding means LavaPurple shows improved MS-compatibility for low abundance protein spots than other fluorescent stains.

During staining with LavaPurple the gels with are basified to approximately pH 10 in order to deprotonate lysine residues. Deprotonation enables the amines to react with the masked aldehyde of epicocconone to produce a stable but highly fluorescent enamine (Figure 2, 2) allowing ultra sensitive detections of proteins (down to picograms) in gels. By lowering the pH to approximately 2.5 after staining, by immersing the gels or blot in 1% citric or 7% acetic acid, epicocconone becomes permanently conjugated to the primary amines and the fluorescent signal is retained for up to 12-months. Raising the pH during tryptic digestion (typically pH 8.5) or Edman degradation results in instability of the protein-fluorophore conjugate and release of unmodified protein or in the case of tryptic digestion unmodified peptides.

In the presence of proteins epicocconone does not need to be maintained at a low pH in order to be fluorescent (Figure 3). This is because an equilibrium is established between conjugated and free epicocconone (Figure 2) such that there is always a high concentration of the fluorescent conjugated form. Thus LavaCell is typically used to stain cell around neutral pH whilst LavaPep operates under mildly alkaline conditions. Fluorescence from the unconjugated form is weak (520 nm) and easily removed with filters.

Epicocconone is excitable by common light sources enabling analysis by standard fluorescence scanners, fluorescence plate readers and CCD camera systems. Another advantage of the spectral characteristics shown in Figure 4 is the large Stokes' shift of epicocconone when bound to proteins (up to 200 nm), which enables simple multiplexing with a wide range of shorter Stokes' shift fluorophores (CyDyes, SYTOX, Hoechst 33342, fluorescein, DAPI, etc) using a single light source [4]. The spectral compatibility of with CyDye DIGE Fluors (Cy2, Cy3, Cy5) allows full integration in the Ettan DIGE workflow.

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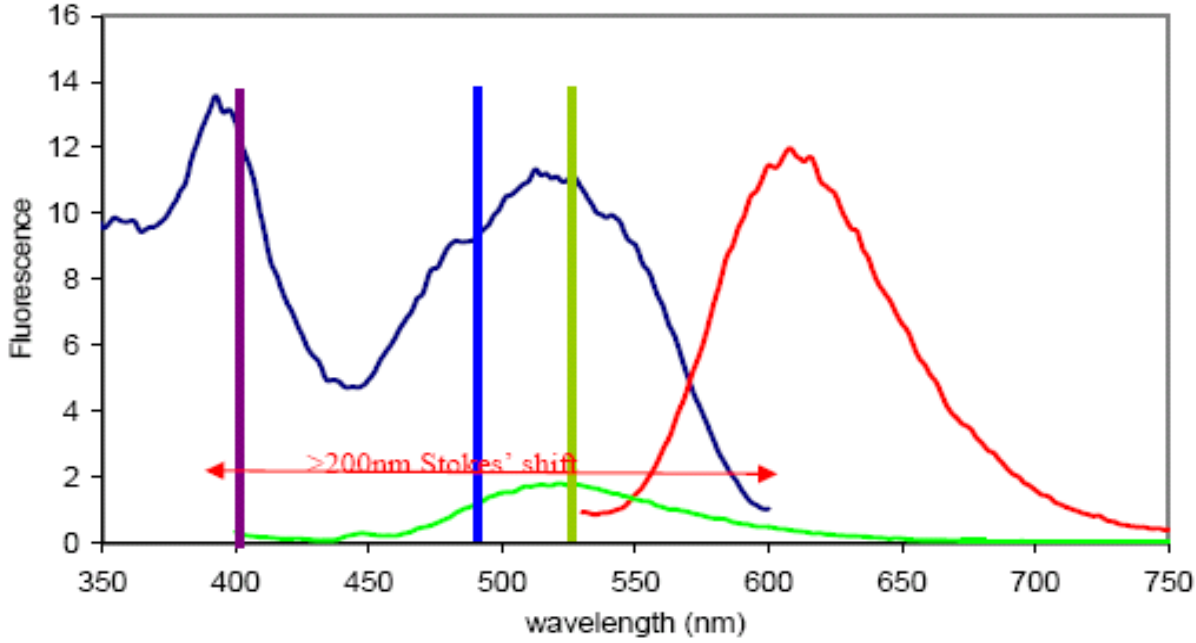

Figure 4. Fluorescence spectral characteristics of epicocconone in water (green) and in the presence of Bovine Serum Albumin (BSA). Vertical lines represent common lasers used to excite the epicocconone-protein adduct (405 nm diode laser, 488 Ar ion laser and 532 frequency double Ng-YAG laser).

References

1. Bell, P.J.L. and Karuso, P. (2003) Epicocconone, a novel fluorescent compound from the fungus Epicoccum nigrum. Journal of the American Chemical Society, 125, 9304.
2. Coghlan, D. R., Mackintosh, J. & Karuso, P. (2005). Mechanism of reversible fluorescent staining of protein with Epicocconone. Organic Letters, 7, 2401-240
3. Mackintosh, J.A., Veal, D.A. and Karuso, P. (2005) FluoroProfile, a fluorescence based assay for rapid and sensitive quantification of proteins in solution. Proteomics, 5, 4673-4677.
4. Choi, H.-Y., Veal, D.A. & Karuso, P. (2006) Epicocconone, A New Cell-Permeable Long Stokes' Shift Fluorescent Stain for Live Cell Imaging and Multiplexing. Journal of Fluorescence, 16 475-482
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6. Mackintosh, J.A., Choi, H.-Y., Bae, S.-H., Veal, D.A., Bell, P.J., Ferrari, B.C., van Dyk, D., Verrills, N.M., Paik, Y.-K. & Karuso, P. (2003). A fluorescent natural product for ultra sensitive detection of proteins in 1-D and 2-D gel electrophoresis, Proteomics. 3, 2273-2288.
7. Tannu, N.S. Sanchez Brambila, G.S., Kirby, P., Andacht, T.M. (2006) Effect of staining reagent on peptide mass fingerprinting from in-gel trypsin digestions: A comparison of Sypro Ruby and LavaPurple. Electrophoresis 27, 3136 - 3143

Legals

Ordering information

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