Fluorescamine

Useful for the fluorometric assay of amino acids, protein, proteolytic enzymes and effectively blocks newly generated amino termini in protein sequence analyses.

Product Information

Name :	**Fluorescamine**
	Spiro(furan-2(3H),1'(3'H)-isobenzofuran) -3,3'-dione, 4-phenyl
	4-Phenyl-4,5-dihydro-3H,3'H-spiro[furan-2,1'-isobenzofuran]-3,3'-dione
Catalog Number :	FP-12631B 250 mg
	FP-R12469 25 mg Pure Grade
Structure :	$C_{17}H_{10}O_4$
Molecular Weight :	278.26
Solubility:	MeCN
Absorption / Emission :	$\lambda_{exc}\backslash\lambda_{em}$ = 390 nm / 465 nm

Storage: -20°C recommended, protected from light and moisture

Introduction

Fluorescamine in not fluorescent, but reacts readily under mild conditions with primary amines in amino acids and peptides to form stable, highly fluorescent compounds. It has to be used as a reagent for the detection of amines and peptides, but requires large excess and suffers from a high rate of hydrolysis, which results in high blanks. Alternatively, the **OPA** method requires the addition of mercaptoethanol to react with amines, and excitation at 340 nm, which causes substantial fluorescence background by almost any biological matter.

Directions for use

Following is a standard protocol. Conditions should be optimized for specific applications, notably the duration of incubation depending on amino acid or aa-content of peptides/proteins.

Protocol 1 : Protein and peptide assay

This assay [Bohlen et al (1974)]may be used to accurately measure protein concentrations between 1-100 µg/mL protein in normal homogenization buffers containing salts and detergents, and even down 10ng protein. It is fast and easy and yields a stable product that can be measured hours later with no loss of signal.

Reagents

0.1 M borate buffer, pH 9.0, containing 1% (w/v) SDS

5 mg Fluorescamine in 10 mL fresh acetone (or acetonitrile can also be used)
Note: The Fluorescamine reagent is stable for 1 week; store at room temperature in the dark.

Bovine serum albumin (BSA; for standards): 1 mg/mL in the buffer you are using

This assay method is compatible with a maximum of 50% acetonitrile, if needed.

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Procedure

Note: always use new test tubes in this assay.

1. Add 1.0 mL borate/SDS to sample (up to 100 µL) in new 10 x 75 mm glass tubes; do not use washed tubes due to the problems of contamination and the sensitivity of this assay. Deliver BSA standards to tubes; use 1 to 100 µL; QS each tube to 100 µL with H_2O . Also include a tube containing 100 µL buffer only.

2. Boil for 5 minutes. Cool to room temperature. If you are dealing with soluble samples, this boiling step may be omitted

3. Add 125 µL of fluorescamine slowly, drop-wise with mixing: Do this addition as the tube is being held on a vortex mixer.

4. Read in a spectrofluorometer (excitation 390 nm; emission 475 nm); mix the contents just before placing the tube in the fluorometer.
Note: This instrument can be fitted with a test tube adapter in the cuvette holder to accommodate the 10 x 75 mm test tubes. Otherwise, rinse the cuvette between each sample.

5. Plot standard curve of BSA (0 to 100 µg). Read samples from standard curve.

References – Fluoresceamine

- **Böhlen P.** , S. Stein,W. Dairman, and S. Undenfriend, Arch. Biochem. Biophys. 155, 1973, 213–220.
- **Jones T.** *et al.*, Failures in Clinical Treatment of *Staphylococcus aureus* Infection with Daptomycin Are Associated with Alterations in Surface Charge, Membrane Phospholipid Asymmetry, and Drug Binding, *Antimicrob. Agents Chemother*., 52: 269 – 278 (2008) Article
- **Maines L.** *et al*., Pharmacologic Manipulation of Sphingosine Kinase in Retinal Endothelial Cells: Implications for Angiogenic Ocular Diseases, *Invest. Ophthalmol. Vis. Sci*., 47: 5022 - 5031 (2006) Article
- **Sánchez-Lozada L.** *et al.*, Treatment with the xanthine oxidase inhibitor febuxostat lowers uric acid and alleviates systemic and glomerular hypertension in experimental hyperuricaemia, *Nephrol. Dial. Transplant.*, 23: 1179 – 1185 (2008) Article
- **Skelley A**. *et al*., Development and evaluation of a microdevice for amino acid biomarker detection and analysis on Mars, PNAS, 102: 1041 - 1046 (2005) Article
- **Wu JJ** *et al.*, Characterization of a core binding site for ADAMTS-13 in the A2 domain of von Willebrand factor, PNAS 103: 18470 - 18474 (2006) Article

Related products

- OPA, FP-02727F (fluorescent detection of Amine and Sulfhydryls)
- FQ derivatization reagent [3-(2-furoyl)quinoline-2-carboxaldehyde)], FP-86524A
- DTNB (Ellman's reagent), UP01566I (chromogenic detection of Sulfhydryls)
- Aminated fluoresceins

5- and 6-AminoFluorescen 126060 (isomer I FP-M1340A and Isomer II (534742)
4-AMF [4'-(Aminomethyl)fluorescein, hydrochloride] FP-M1161
5-DTAF [5-(4,6-Dichlorotriazinyl)aminofluorescein] FP-46732A
DAF-2 DA[4,5-Diaminofluorescein Diacetate] FP-F9657A and its diacetate form DAF-2 DA FP-S0372A
Fluorescein cadaverine, diHBr FP-46576B
5-DTAF [5-(4,6-Dichlorotriazinyl)aminofluorescein] FP-46732A
6-Carboxy-4'-Aminomethylfluorescein FP-JQ4361
Fluorescein PEG Amine [FITC-PEG-NH2, MW 3400] DY2720
APF [Aminophenyl Fluorescein] CA7270

Ordering information

Catalog size quantities and prices may be found at http://www.fluoprobes.com
Please inquire for higher quantities (availability, shipment conditions).

For any information, please ask : FluoProbes® / Interchim; Hotline : +33(0)4 70 03 73 06

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