

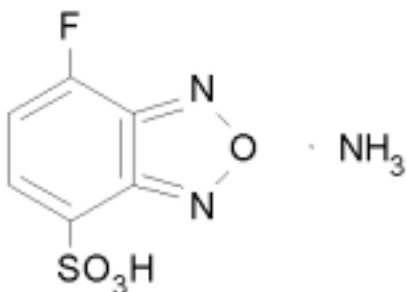
7-FLUOROBENZO-2-OXA-1,3-DIAZOLE-4-SULFONIC ACID AMMONIUM

 Product Number **F4383**

Storage Temperature RT

CAS #: 84806-27-9

 Synonyms: SBD-F¹, 4-Fluoro-7-Sulfobenzofurazan, Ammonium salt²,

Product Description

 Appearance: White to light yellow powder³

 Molecular Formula: C₆H₃FN₂O₄S·NH₃

Molecular Weight: 235.2

 Method of preparation of SBD-F was reported.¹

SBD-F is a sensitive and specific fluorescent labeling reagent for low molecular weight compounds and for macromolecules containing thiol groups.^{1,4-10} SBD-F reacts with sulfhydryl groups (disulfides do not react with SBD-F and must first be reduced to thiols, for example using tributylphosphine)¹⁰ to produce highly fluorescent compounds. SBD-F does not exhibit any fluorescence of its own and no fluorescent by-products are formed in the reaction with SBD-F.^{1,8} Suggested optimal reaction conditions for formation of fluorescent derivatives are SBD-F (0.5 mM), 60°C, and pH 9.5 (0.1 M borate buffer containing 1 mM EDTA disodium) for one hour.¹ Additional procedures have been reported.¹⁰ At pH 9.5, the inclusion of 1 mM EDTA in the buffer is important to prevent metal-catalyzed oxidation of thiols.¹⁰ The rate of reaction of thiols with SBD-F gradually increases with increasing pH.¹⁰ High fluorescence intensities are observed at pH 2-12 except for SBD-cysteine and SBD- (thiol containing amino acids) which require an acidic (pH 2) medium.¹⁰

For the SBD-F derivative:

 Excitation wavelength: 380-385nm^{1,4,5}

 Emission wavelength: 515nm^{1,4,5}, 510nm⁶

Product Information

SBD-F is suitable for the HPLC determination of biological thiols at the picomole level.¹ The detection limit of thiol compounds, glutathione, coenzyme A, cysteamine, homocysteine, N-acetylcysteine, cysteine and D-3-mercaptopropionyl-L-proline (captopril) were 100, 120, 160, 330, 390, 3600, 150 pmol/ml, respectively¹. The amino acids, proline and alanine (contain no -SH groups), did not give any fluorescence with SBD-F. Some thiols (cysteine, glutathione and captopril) have also been determined by reaction with SBD-F then separated and quantified by HPLC.^{7,10} The determination of SBD-thiols (both reduced and oxidized) in plasma by HPLC has been reported.^{4,7} SBD-F has been used in HPLC methods for measuring total plasma and serum homocysteine levels.^{4,5,8-10} SBD-F has been used in the determination of metallothionein in a tandem column HPLC method with an isocratic solvent system.⁶ SBD-F has been used for determining the position of disulfide linkages of cysteine residues in proteins¹⁰⁻¹² and for the detection of cystine containing peptides.¹³ Positions of disulfide bonds in Yam acidic class IL (class IV) chitinase were determined by digestion of the chitinase. The resulting disulfide bonds containing peptides were separated by reversed-phase HPLC and detected using SBD-F¹¹; SBD-F was employed in the determination of disulfide bridges in Factor Va heavy chain.¹²

Storage/Stability

The product is stable for 3 years when stored at room temperature and protected from light.³

Preparation Instructions

SBD-F has been solubilized in 1 M ammonium hydroxide at 50 mg/ml yielding a clear yellow to yellow-green solution.³ The product is also freely soluble in deionized water. A 10 mg/ml solution has been prepared.¹ SBD-F solutions in water were reported to be stable for more than one week at 2-8°C.¹ SBD-F derivatives were stable at pH 9.5¹ for at least one week at room temperature when stored in the dark^{1,5,6,9} and 0°C¹⁰ (derivatized samples may turn yellow because of the light sensitivity of SBD-F. Use of the derivatives did not seem to affect the assay for homocysteine determination in plasma).⁹ SBD-F homocysteine complexes are more stable than o-phthalaldehyde (OPA)-homocysteine complexes when both were stored in the dark.⁵

References

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