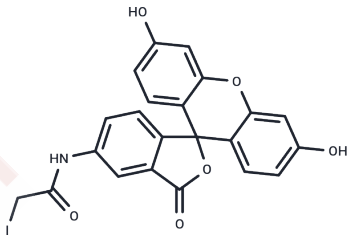
Data Sheet (Cat.No.T14052)

TargetMol

5-IAF

Chemical Properties



CAS No. :	63368-54-7
Formula:	C22H14INO6
Molecular Weight:	515.25
Storage:	Store at low temperature,Keep away from direct sunlight Powder: -20°C for 3 years | In solvent: -80°C for 1 year Actual storage temperature shall be subject to the COA.

Biological Description

Description	5-IAF is a fluorescein derivative of indoleacetamide, a fluorescent probe that can be used to label proteins and others containing free thiols (cysteine side chains). 5-IAF can label the catalytic (alpha) subunit of dog kidney Na,K-ATPase.
Targets(IC50)	Others
In vitro	Instructions 1. Preparation and storage a. Solubility: 5-IAF is usually provided in powder form and is easily soluble in DMSO, DMF or aqueous solution at pH ≥ 8. Recommended stock solution concentration: 10–50 mM (adjust according to experimental needs). Before use, dissolve it in anhydrous solvent and aliquot into small volumes to avoid repeated freezing and thawing. b. Storage conditions: Powder: Store in a dry environment, away from light at -20°C or lower. Solution: Short-term storage at 4°C, long-term storage at -20°C away from light is recommended. 2. Thiol labeling a. Protein reduction treatment (if necessary): To ensure that the thiol groups are in a reduced state, protein samples can be pretreated with DTT or TCEP. Use a thiol-free buffer (such as PBS or HEPES), pH 7–8 is the optimal range. b. Labeling reaction: Dilute the stock solution of 5-IAF to an appropriate working concentration (usually 0.1–1 mM). Add to the protein solution (usually 1–10 mg/mL) and mix gently. c. Incubate at room temperature for 30 minutes to 2 hours, the specific time needs to be optimized according to the experiment. d. Termination reaction: Terminate the labeling reaction by adding excess reducing agent (such as β-mercaptoethanol) or dilution. e. Purification: Remove unbound 5-IAF and reaction byproducts using dialysis, gel filtration, or centrifugal ultrafiltration. 3. Fluorescence detection Excitation wavelength: ~495 nm. Emission wavelength: ~520 nm (green fluorescence). The fluorescent signal of the labeled protein can be detected in a fluorescence

In vitro	microscope or fluorescence spectrometer. The above information is based on published literature. Experimental procedures should be appropriately modified to meet specific research demands.

Solubility Information

Solubility	DMF: 90 mg/mL (174.67 mM),Sonication and heating to 60℃ are recommended. DMSO: 45 mg/mL (87.34 mM),Sonication is recommended. (< 1 mg/ml refers to the product slightly soluble or insoluble)

Preparing Stock Solutions

	1mg	5mg	10mg
1 mM	1.9408 mL	9.704 mL	19.4081 mL
5 mM	0.3882 mL	1.9408 mL	3.8816 mL
10 mM	0.1941 mL	0.9704 mL	1.9408 mL
50 mM	0.0388 mL	0.1941 mL	0.3882 mL

Please select the appropriate solvent to prepare the stock solution, according to the solubility of the product in different solvents. Please use it as soon as possible.

Note: The dilution table applies only to solid products. For liquid products, please calculate the stock solution based on the stated concentration and/or density.

Reference

Wang Y, et al. Determination of free and protein-bound glutathione in HepG2 cells using capillary electrophoresis with laser-induced fluorescence detection. J Chromatogr A. 2009 Apr 17;1216(16):3533-7.
Indo HP,et al. Manganese superoxide dismutase promotes interaction of actin, S100A4 and Talin, and enhances rat gastric tumor cell invasion. J Clin Biochem Nutr. 2015 Jul;57(1):13-20.
Clark HM, Hagedorn TD, Landino LM. Hypothiocyanous acid oxidation of tubulin cysteines inhibits microtubule polymerization. Arch Biochem Biophys. 2014 Jan 1;541:67-73.