5-IAF

Cat. No.: HY-D0807

CAS No.: 63368-54-7

Molecular Formula: $C_{22}H_{14}INO_6$

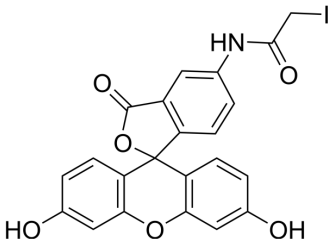
Molecular Weight: 515.25

Target: Fluorescent Dye

Pathway: Others

Storage: -20°C, protect from light

* In solvent : -80°C, 6 months; -20°C, 1 month (protect from light)



SOLVENT & SOLUBILITY

In Vitro

DMSO : 50 mg/mL (97.04 mM; Need ultrasonic)

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	1.9408 mL	9.7040 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

Please refer to the solubility information to select the appropriate solvent.

In Vivo

1. Add each solvent one by one: 10% DMF >> 40% PEG300 >> 5% Tween-80 >> 45% saline
   Solubility: ≥ 2.5 mg/mL (4.85 mM); Clear solution
2. Add each solvent one by one: 10% DMF >> 90% (20% SBE-β-CD in saline)
   Solubility: 2.5 mg/mL (4.85 mM); Suspended solution; Need ultrasonic
3. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
   Solubility: 1.43 mg/mL (2.78 mM); Suspended solution; Need ultrasonic
4. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: 1.43 mg/mL (2.78 mM); Suspended solution; Need ultrasonic

BIOLOGICAL ACTIVITY

Description

5-IAF (5-Iodoacetamidofluorescein) is an idoacetamide derivate of fluoresceine. 5-IAF can be used as fluorescent probe that labels proteins and other molecules having free thiols (cysteine side chains)[1][2].

In Vitro

8 μM GSH sample solution is derivatized with 8, 80, 200, 400, 800, 1600, 2000, 2500 μM 5-IAF, respectively and analyzed with CE-LIF. The highest signal intensity is obtained with 400 and 800 μM 5-IAF[2].

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Kinase Assay [2]

HepG2 cells are lysed in 1 mL 2% acetonitrile in water, and transfered to a 1.5 mL centrifuge tube. An additional 200 µL 2% acetonitrile is used to rinse the dish and combined with the cell extract. To measure the free GSH concentration, three aliquots of 80 µL cell extract are immediately transferred to 0.5 mL centrifuge tubes after a quick mix. Then 20 µL 50 µM NAC is added, followed by the addition of 100 µL 400 µM 5-IAF to the mixture. The derivatization is conducted at room temperature in dark for 1 h, after which the mixtures are centrifuged at 9000× g for 5 min to sediment the insoluble proteins. 10 µL supernatant is diluted 100× with water and analyzed immediately by CE-LIF[2].

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

REFERENCES

[1]. Salvatore Sotgia, et al. Plasma L-Ergothioneine Measurement by High-Performance Liquid Chromatography and Capillary Electrophoresis after a Pre-Column Derivatization with 5-Iodoacetamidofluorescein (5-IAF) and Fluorescence Detection. PLoS One. 2013; 8(7): e70374.

[2]. Yan Wang, 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.

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