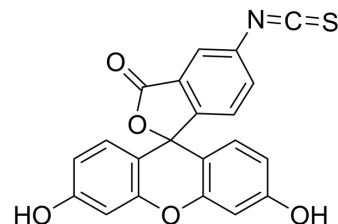


## FITC

<b>Cat. No.:</b>	HY-66019
<b>CAS No.:</b>	3326-32-7
<b>Molecular Formula:</b>	C <sub>21</sub> H <sub>11</sub> NO <sub>5</sub> S
<b>Molecular Weight:</b>	389.38
<b>Target:</b>	Fluorescent Dye
<b>Pathway:</b>	Others
<b>Storage:</b>	-20°C, protect from light, stored under nitrogen * In solvent : -80°C, 6 months; -20°C, 1 month (protect from light, stored under nitrogen)



## SOLVENT & SOLUBILITY

<b>In Vitro</b>	DMSO : 50 mg/mL (128.41 mM; Need ultrasonic)			
	H <sub>2</sub> O : < 0.1 mg/mL (insoluble)			
		Solvent Concentration	Mass	
			1 mg	5 mg
<b>Preparing Stock Solutions</b>	<b>1 mM</b>	2.5682 mL	12.8409 mL	25.6819 mL
	<b>5 mM</b>	0.5136 mL	2.5682 mL	5.1364 mL
	<b>10 mM</b>	0.2568 mL	1.2841 mL	2.5682 mL
Please refer to the solubility information to select the appropriate solvent.				
<b>In Vivo</b>	1. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline Solubility: ≥ 2.08 mg/mL (5.34 mM); Clear solution  2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline) Solubility: 2.08 mg/mL (5.34 mM); Suspended solution; Need ultrasonic			

## BIOLOGICAL ACTIVITY

<b>Description</b>	<p>FITC (Fluorescein Isothiocyanate), is one of the green fluorescein derivatives widely used in biology. FITC has the characteristics of high absorptivity, excellent fluorescence quantum yield and good water solubility. The isothiocyanate group of FITC can be combined with amino, sulfhydryl, imidazole, tyrosyl, carbonyl and other groups on the protein, so as to achieve protein labeling including antibodies and lectins. In addition to its use as a protein marker, FITC can also be used as a fluorescent protein tracer to rapidly identify pathogens by labeling antibodies, or for microsequencing of proteins and peptides (HPLC). The maximum excitation wavelength of FITC is 494 nm. Once excited, it fluoresces yellow-green at a maximum emission wavelength of 520 nm.</p>
<b>In Vitro</b>	Protocol

### 1. Protein Preparation

- 1) In order to obtain the best labeling effect, please prepare the protein (antibody) concentration as 2 mg/mL.
- 2) The pH value of protein solution shall be 8.5±0.5. If the pH is lower than 8.0, 1M sodium bicarbonate shall be used for adjustment.
- 3) If the protein concentration is lower than 2mg/ml, the labeling efficiency will be greatly reduced. In order to obtain the best labeling efficiency, it is recommended that the final protein concentration range is 2-10 mg/mL.
- 4) The protein must be in the buffer without primary amine (such as Tris or glycine) and ammonium ion, otherwise the labeling efficiency will be affected.

### 2. Dye Preparation

Add anhydrous DMSO into the vial of FITC to make a 10 mM stock solution. Mix well by pipetting or vortex.

### 3. Calculation of dye dosage

The amount of FITC required for reaction depends on the amount of protein to be labeled, and the optimal molar ratio of FITC to protein is about 10.

Example: assuming the required marker protein is 1 mL 2 mg/mL IgG (MW=150,000), use 1 mL DMSO dissolve 1 mg FITC, the required FITC volume is 40 µL.

### 4. Run conjugation reaction

- 1) A good volume of freshly prepared 10 mg/mL FITC is slowly added to 0.5 mL protein sample. In solution, gently shake to mix, then centrifuge briefly to collect the sample at the bottom of the reaction tube. Don't mix well to prevent protein samples from denaturation and inactivation.
- 2) The reaction tubes were placed in a dark place and incubated gently at room temperature for 60 minutes at intervals. For 10-15 minutes, gently reverse the reaction tubes several times to fully mix the two reactants and raise the bar efficiency.

### 5. Purify the conjugation

The following protocol is an example of dye-protein conjugate purification by using a Sephadex G-25 column.

- 1) Prepare Sephadex G-25 column according to the manufacture instruction.
- 2) Load the reaction mixture (From "Run conjugation reaction") to the top of the Sephadex G-25 column.
- 3) Add PBS (pH 7.2-7.4) as soon as the sample runs just below the top resin surface.
- 4) Add more PBS (pH 7.2-7.4) to the desired sample to complete the column purification.

Combine the fractions that contain the desired dye-protein conjugate.

### Note

1. FITC is sensitive to light and humidity. Immediately add FITC solution and discard the unused part.
2. Low concentrations of sodium azide ( $\leq 3$  mM or 0.02%) or thiomersal ( $\leq 0.02$  mM or 0.01%) did not significantly interfere with protein labeling; However, 20-50% glycerol will reduce labeling efficiency.
3. Avoid buffering with primary amines (e.g., Tris, glycine) or ammonium ions, It compete with labeled proteins.
4. This product is only for scientific research by professionals, and shall not be used in clinical diagnosis or treatment, food or medicine.
5. For your safety and health, please wear lab coat and disposable gloves.

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

## PROTOCOL

### Cell Assay <sup>[1]</sup>

The frequency of chondrocyte apoptosis is measured by flow cytometry with Annexin V-fluorescein isothiocyanate (FITC) and propidium iodide. A total of  $1 \times 10^4$  treated chondrocytes are collected from each group, washed in cold PBS and incubated with Annexin V-FITC and PI at room temperature for 15 min in the dark on ice. These samples are then analyzed using a fluorescence-activated cell sorter. Cell Quest software is used to analyze the percentage of apoptosis. All tests are repeated in triplicate.

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

## CUSTOMER VALIDATION

- Adv Mater. 2024 Feb 1:e2313248.
- Adv Mater. 2023 Sep 5;e2306469.
- Adv Funct Mater. 2024 Feb 8.
- Sci Bull. 2022 Dec 30;S2095-9273(22)00609-0.
- ACS Nano. 2023 Jul 10.

See more customer validations on [www.MedChemExpress.com](http://www.MedChemExpress.com)

## REFERENCES

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[1]. Gao SG, et al. Phosphorylation of osteopontin has proapoptotic and proinflammatory effects on human knee osteoarthritis chondrocytes. Exp Ther Med. 2016 Nov;12(5):3488-3494. Epub 2016 Oct 5.

[2]. Gao SG, et al. Phosphorylation of osteopontin has proapoptotic and proinflammatory effects on human knee osteoarthritis chondrocytes. Exp Ther Med. 2016 Nov;12(5):3488-3494. Epub 2016 Oct 5.

[3]. Zhu X, et al. Ratiometric, visual, dual-signal fluorescent sensing and imaging of pH/copper ions in real samples based on carbon dots-fluorescein isothiocyanate composites. Talanta. 2017 Jan 1;162:65-71

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**Caution: Product has not been fully validated for medical applications. For research use only.**

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