

5-Iodoacetamidofluorescein (5-IAF)

62246

2243.0

Number

62246

Description**5-Iodoacetamidofluorescein (5-IAF), 25 mg**

Molecular weight: 515.2

Excitation wavelength: 494 nm

Emission wavelength: 518 nm

Molar extinction coefficient at 492 nm: 80,000-85,000 M⁻¹ cm⁻¹Formula: C₂₂H₁₄INO₆

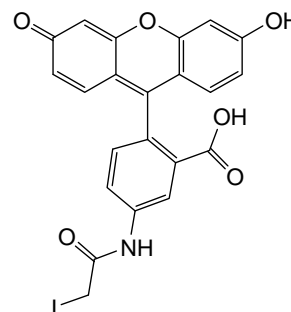
CAS # 63368-54-7

CAS name: Acetamide, *N*-(3',6'-dihydroxy-3-oxospiro(isobenzofuran-1(3H), 9'-(9H)xanthen)-5-yl)-2-iodo

Form and color: solid, yellow-orange

Solubility: Dimethyl formamide (DMF) and aqueous buffers above pH 6

Purity: > 90% by HPLC

Storage: Upon receipt store product at -20°C protected from light. Product is shipped at ambient temperature.**Introduction**

5-Iodoacetamidofluorescein (5-IAF) is a fluorescein derivative that contains an iodoacetamido group for labeling proteins, peptides and other biomolecules. Iodoacetamides primarily react with sulfhydryl groups to form stable thioether bonds at physiological pH and at room temperature or below. If free sulfhydryls are not present, iodoacetamides can react with methionine, histidine or potentially tyrosine. At pH < 8, most aliphatic amines are protonated and nonreactive to iodoacetamides. 5-IAF is soluble in buffers at pH > 6 and has been widely used as a tracer in living cells and in the study of the structural properties, diffusion and interactions of proteins, especially in muscle. 5-IAF has been conjugated to many different proteins including actin, myosin, troponin, hemoglobin, and thiol-containing proteins in SDS gels.

Important Product Information

- Store product in the original container at -20°C protected from light. Equilibrate vial to room temperature before opening to avoid moisture condensation onto the product. 5-IAF is unstable in light, especially in solution. Prepare this reagent immediately before use. Do not store this reagent in aqueous solutions.
- 5-IAF reacts with free -SH group(s). Some sulfhydryl-containing peptides and proteins may oxidize in solution and form disulfide bonds, which cannot react. Disulfide bonds can be reduced to produce free sulfhydryls. The Immobilized TCEP Disulfide Reducing Gel (Product No. 77712) enables peptide or protein reduction while recovering the sample in the absence of reducing agents.
- As an alternative to disulfide reduction, sulfhydryls can be introduced via amine modification using *N*-succinimidyl *S*-acetylthioacetate (SATA, Product No. 26102) or 2-iminothiolane•HCl (Traut's Reagent, Product No. 26101).

Example Procedure for IgG Reduction and Labeling

This protocol is an example application for 5-IAF and, therefore, specific applications will require optimization. In this method, whole IgG is reduced with 2-MEA, which cleaves disulfide bonds between antibody heavy chains while preserving those between heavy and light chains. During reduction, absolute concentration of 2-MEA is more critical than antibody concentration because 1-10 mg IgG can be effectively reduced with 50 mM of 2-MEA. This protocol can be modified for other disulfide-containing molecules.

Note: EDTA added to buffers prevents metal-catalyzed oxidation of sulfhydryls.

A. Materials Required

- 2-Mercaptoethylamine•HCl (2-MEA, Product No. 20408)
- Reducing Buffer: Prepare 1 ml of buffer by combining 100 μ l of 1 M sodium phosphate pH 6.0, 5 μ l of 0.5 M EDTA and 900 μ l ultrapure water.
- Phosphate-buffered saline (PBS): 0.1 M phosphate, 0.15 M sodium chloride; pH 7.2 (Product No. 28372) or other buffer at pH 6.5-7.5
- Conjugation Buffer: Add 20 μ l of 0.5 M EDTA to 10 ml of PBS for each 10 ml of Conjugation Buffer needed.
- 37°C incubator or water bath
- Desalting column, such as Thermo Scientific Dextran Desalting Columns (Product No. 43230) for removing 2-MEA. For removing excess 5-IAF, use either a desalting column or a Thermo Scientific Slide-A-Lyzer Dialysis Cassette.
- Dimethyl formamide (DMF, Product No. 20673)
- IgG Solution: Dissolve 1-10 mg of IgG in 1 ml of Reducing Buffer.

B. Reduction of IgG Disulfide Bonds

1. Add the 1 ml IgG Solution to a 6 mg vial of 2-MEA and gently shake vial to dissolve.
2. Incubate reaction for 90 minutes at 37°C.
3. Cool the solution to room temperature.
4. Remove 2-MEA from the reduced antibody using a desalting column equilibrated with Conjugation Buffer. After the antibody solution has entered the gel bed, add additional Conjugation Buffer and collect 500 μ l fractions.
5. Determine antibody location by measuring the absorbance of each fraction at 280 nm. Pool fractions containing reduced antibody. Proceed immediately to Section C to minimize sulfhydryl oxidation.

C. Labeling of Reduced IgG

Note: Upon protein reduction or modification, for best results remove excess reducing or modification reagent by desalting before reacting with the 5-IAF.

1. Dissolve 1 mg 5-IAF in 100 μ l of DMF. Determine the volume of 5-IAF needed for a 5- to 10-fold molar excess over IgG and add it to the tube containing the reduced IgG solution.
2. Mix the reaction well and allow it to proceed for 2 hours in the dark at room temperature.
3. Remove non-reacted 5-IAF from the antibody by desalting or dialysis.
4. Store labeled antibody protected from light at 4°C for up to one month. Alternatively, store labeled antibody in single-use volumes at -20°C.

Example Procedure for Labeling Protein in the Presence of DTT

The following protocol, adapted from Gorman (1987), will require optimization for each protein. The protocol uses DTT to reduce disulfide bonds to produce free thiols followed by reaction with an excess of 5-IAF. Because the protocol uses an excess of 5-IAF over the DTT, removal of DTT is not necessary to achieve adequate conjugation.

1. Dissolve protein containing disulfide bonds at 5-10 mg/ml in 0.1 M NH_4HCO_3 containing 1% SDS and 20 mM dithiothreitol (DTT).
2. Incubate for 16 hours at 0°C or 2 hours at 22°C.
3. Add a five-fold molar excess of 5-IAF over DTT. Incubate for 2 hours in the dark at 22°C.
4. Maintain the pH at 7.5-8.0 by adding 6 M NaOH as necessary. Typically, adding 2 μl per 100 mM of 5-IAF will adequately adjust the pH.
5. Precipitate protein by adding nine volumes of HPLC-grade methanol at -20°C. Collect protein by centrifuging at 4°C at $8,000 \times g$ for 5 minutes.

Calculate the Degree of Labeling (F/P)

Note: Non-reacted dye must be completely removed for accurate determination of the dye-to-protein ratio (F/P).

1. Measure the absorbance of the protein:dye conjugate at 280 nm using a spectrophotometer cuvette that has a 1 cm path length. If initial absorbance measurement is > 2 , dilute sample, or a aliquot thereof, by a factor necessary to obtain absorbance values less than 2.0. Record the dilution factor, which will be required in the calculation
2. Calculate molarity of the protein:
 - ϵ = protein molar extinction coefficient (e.g., the molar extinction coefficient of IgG is $\sim 210,000 \text{ M}^{-1} \text{ cm}^{-1}$)
 - $A_{\text{max}} = A_{491}$
 - CF = Correction factor = $\frac{A_{280}}{A_{\text{max}}}$; adjusts for the absorbance amount 5-IAF will have at 280 nm

Note: if the sample amount is limited, a correction factor of 0.3 may be used for 5-IAF.

$$\text{Protein concentration (M)} = \frac{A_{280} - (A_{\text{max}} \times \text{CF})}{\epsilon} \times \text{dilution factor}$$

3. Calculate the degree of labeling:
 - ϵ' = 5-IAF molar extinction coefficient: use $82,000 \text{ M}^{-1} \text{ cm}^{-1}$

$$\text{Moles dye per mole protein} = \frac{A_{\text{max}} \text{ of the labeled protein}}{\epsilon' \times \text{protein concentration (M)}} \times \text{dilution factor}$$

Additional Information

Visit our web site for additional information relating to this product including the following items:

- Tech Tip #31: Calculate dye:protein (F/P) molar ratios
- Tech Tip #43: Protein stability and storage
- Tech Tip #20: An overview of dialysis
- Tech Tip #6: Extinction coefficients guide
- Tech Tip #30: Modify and label oligonucleotide 5' phosphate groups

Related Thermo Scientific Products

26101	Traut's Reagent, 500 mg
26102	SATA (<i>N</i> -succinimidyl <i>S</i> -acetylthioacetate), 50 mg
26103	Hydroxylamine Hydrochloride, 25 g
77712	Immobilized TCEP Disulfide Reducing Gel, 5 ml
20291	DTT, No-Weigh™ Format, 48 × 7.7 mg microtubes
20408	2-Mercaptoethylamine•HCl, 6 × 6 mg
20490	TCEP•HCl, 1 g
46200	DyLight™ 547 NHS Ester, 1 mg, for labeling at primary amino groups
46205	DyLight 647 NHS Ester, 1 mg, for labeling at primary amino groups

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