

Fluorescein-5-Maleimide

62245

2242.2

Number

62245

Description

Fluorescein-5-Maleimide, 25mg

Formula: C₂₄H₁₃NO₇

Molecular Weight: 427.4

Excitation Wavelength: 494nm

Emission Wavelength: 518nm

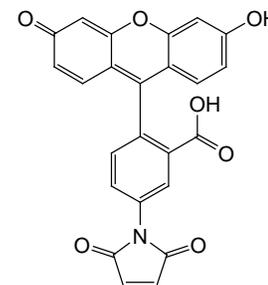
Fluorescein Molar Extinction Coefficient: $\geq 80,000\text{M}^{-1}\text{cm}^{-1}$

CAS # 75350-46-8

Purity: > 99% by HPLC

Form and color: Solid, yellow-orange

Solubility: Dimethyl formamide



Storage: Upon receipt store desiccated at -20°C. Product is shipped at ambient temperature.

Introduction

The Thermo Scientific™ Fluorescein-5-Maleimide is effective for labeling sulfhydryl-containing molecules. Maleimide groups react predominantly with sulfhydryls at pH 6.5-7.5 forming a stable thioether bond. At pH 7, the maleimide group is ~1000 times more reactive toward a free sulfhydryl than to an amine. At pH values > 7.5, reactivity toward primary amines increases and hydrolysis of the maleimide group can occur.

Important Product Information

- Maleimide groups are moisture-sensitive. Store product in the original container at -20°C with desiccant. Equilibrate vial to room temperature before opening to avoid moisture condensation onto the product.
- Prepare this reagent immediately before use. Do not store these reagents in aqueous solutions.
- Molecules to be labeled with Fluorescein-5-Maleimide must have free -SH group(s) available. Some sulfhydryl-containing molecules may oxidize in solution and form disulfide bonds, which cannot react with maleimides. Disulfide bonds can be reduced to produce free sulfhydryls. After reduction, most reducing reagents must be removed before conjugation. The Thermo Scientific™ Immobilized TCEP Disulfide Reducing Gel (Product No. 77712) enables disulfide 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).
- Avoid sulfhydryl-containing components during conjugation, as these will react with the maleimide portion of the reagent, thereby inhibiting and reducing conjugation efficiency of the intended molecule.

Procedure for Labeling Sulfhydryl-containing Molecules

The following protocol requires optimization for each specific application.

A. Label the Protein

1. Prepare sample with available sulfhydryls in 20mM sodium phosphate buffer, 150mM NaCl, pH 7.2 or other suitable buffer at pH 6.5-7.5. To prevent metal-catalyzed formation of disulfide bonds, add EDTA at a final concentration of 5-10mM.
2. Add a molar excess of Fluorescein-5-Maleimide over the molar amount of sulfhydryl to be coupled. Generally, using a 25-fold molar excess for the Fluorescein-5-Maleimide produces acceptable results.
Note: Fluorescein-5-Maleimide can be directly added to aqueous solution (up to 1mM concentration) or first dissolved at a concentration of 10mM in DMF before adding to the protein solution.
3. Allow the reaction to proceed for 2 hours at room temperature or overnight at 4°C.
4. Remove nonreacted fluorescein by processing with the Thermo Scientific™ Dye Removal Columns (Product No. 22858) or by desalting or dialysis using a 10K molecular-weight cutoff or larger.
5. Store labeled protein or peptide protected from light at 4°C for up to one month. Alternatively, store labeled molecule in single-use aliquots at -20°C.

B. Calculate the Degree of Labeling

Note: The non-reacted fluorescein must be completely removed for optimal results and accurate determination of the fluor-to-protein ratio. It may be necessary to dilute a small amount of the desalted/dialyzed sample.

1. Using a 1 cm path length cuvette, measure the absorbance of the labeled protein at 280 nm and 495 nm.
2. Calculate the concentration of the protein:
 - ϵ = protein molar extinction coefficient (e.g., the molar extinction coefficient of IgG is $\sim 210,000 \text{ M}^{-1} \text{ cm}^{-1}$)
 - $A_{\text{max}} = A_{495}$
 - CF = correction factor = $\frac{A_{280}}{A_{\text{max}}} = 0.3000$

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

3. Calculate the degree of labeling:
 - ϵ' = fluor molar extinction coefficient: $68,000 \text{ M}^{-1} \text{ cm}^{-1}$

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

Troubleshooting

Problem	Cause	Solution
The application in which the labeled protein was used was unsuccessful	The protein was not labeled	Before troubleshooting, determine if the protein is labeled by calculating the $A_{\text{max}}:A_{280}$ ratio – determine this ratio after thorough desalting or dialysis Note: For fluor-labeled antibodies the $A_{\text{max}}:A_{280}$ ratio should be > 1.
The protein was not labeled	Substance interfered with the reaction or incorrect reaction conditions	Ensure that the Conjugation Buffer is at pH 6-7 and does not contain free thiols, such as reducing agents
	There are no free sulfhydryls available on the protein	Reduce existing disulfide bonds to generate free sulfhydryls, or introduce sulfhydryls with Traut's Reagent or SATA

Visit our Website for Additional Information

- 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

22858	Dye Removal Columns
26101	Traut's Reagent, 500mg
26102	SATA (N-succinimidyl S-acetylthioacetate), 50mg
26103	Hydroxylamine Hydrochloride, 25g
77712	Immobilized TCEP Disulfide Reducing Gel, 5mL
20291	Dithiothreitol (DTT), No-Weigh™ Format, 48 × 7.7mg microtubes
20408	2-Mercaptoethylamine•HCl (β-ME), 6 × 6mg
20490	TCEP•HCl, 1g
46100	NHS-Fluorescein, 100mg
46602	DyLight™ 488 Maleimide, 1mg
46607	DyLight 549 Maleimide, 1mg
46615	DyLight 649 Maleimide, 1mg
46618	DyLight 680 Maleimide, 1mg

General References

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