

Maleimide Conjugation Protocol for Thiol Dyes

Description

Thiol dyes are extensively used in biochemical and molecular biology applications to label proteins, peptides, and other biomolecules through their thiol groups. These dyes contain sulfhydryl groups (-SH) that can react specifically with maleimide groups to form stable thioether bonds. This labeling technique is crucial for fluorescence microscopy, flow cytometry, and protein tracking, providing precise visualization and quantification of biomolecules. The following protocol offers a detailed guide for the efficient and effective conjugation of thiol dyes to maleimide-functionalized target molecules, ensuring optimal reaction conditions and storage methods to maintain the integrity and functionality of the conjugate.

| Product name | λ_{Ex} (nm) | λ_{Em} (nm) | Packing Unit | Catalog number |
|------------------------------------|------------------------|------------------------|----------------------------|----------------|
| TAMRA Thiol | 551 | 573 | 1, 5, 25 mg | KWT1057 |
| ICG Thiol | 785 | 812 | 1, 5, 25 mg | PWT1301 |
| FAM Thiol | 494 | 520 | 1, 5, 25 mg | CWT1003 |
| Flamma [®] 496 Thiol | 496 | 520 | 1, 5, 25 mg | CWT1001 |
| Flamma [®] 552 Thiol | 550 | 564 | 1, 5, 25 mg | CWT1058 |
| Flamma [®] 581 Thiol | 578 | 593 | 1, 5, 25 mg | KWT1415 |
| Flamma [®] 648 Thiol | 648 | 663 | 1, 5, 25 mg | KWT1042 |
| Flamma [®] 675 Thiol | 675 | 691 | 1, 5, 25 mg | PWT1415 |
| Flamma [®] 749 Thiol | 749 | 774 | 1, 5, 25 mg | PWT1215 |
| Flamma [®] 774 Thiol | 774 | 800 | 1, 5, 25 mg | PWT1515 |
| qFlamma [®] Black01 Thiol | 484* | | * Absorption range 400~800 | QWT1001 |

Materials and Preparation

- **Thiol Dye Stock Solution:** Prepare a 10 mM solution of the thiol dye in anhydrous DMSO or DMF. Briefly vortex to mix and store any unused solution in the dark at -20 °C.
- **Maleimide-Functionalized Target Molecule Solution:** Dissolve the maleimide-functionalized target molecule (protein, peptide, etc.) at a concentration of 1-10 mg/mL in a degassed buffer (such as PBS, Tris, or HEPES at pH 7.0-7.5). Ensure the buffer does not contain any competing thiols.
- **Reducing Agent (Optional):** If the target molecule contains disulfide bonds that need to be reduced, add a 10-100× molar excess of TCEP to the solution, flush with inert gas, and incubate for 20-30 minutes at room temperature. Alternatively, DTT can be used, but excess DTT must be removed via dialysis before conjugation.

Conjugation Process

1. Dye Addition: Combine the thiol dye solution with the maleimide-functionalized target molecule solution at a molar ratio of 10-20× excess dye to target molecule. Stir gently or vortex to mix.
2. Reaction Conditions: Flush the vial with inert gas, seal tightly, and protect from light. Incubate the mixture at room temperature for 2 hours or overnight at 2-8 °C.

Purification

- Remove Excess Dye: Remove unreacted dye using a suitable purification method such as gel filtration, dialysis, HPLC, FPLC, or electrophoresis, depending on the target molecule and the specific requirements of your experiment.

Storage

- Short-Term Storage: For best results, use the purified conjugate immediately. If needed, store the conjugate at -20 °C, protected from light, for up to one week.
- Long-Term Storage: For extended storage, add 5-10 mg/mL BSA and 0.01-0.03% sodium azide to prevent denaturation and microbial growth. Optionally, add 50% glycerol and store at -20 °C for up to a year.

Degree of Labeling Calculation

- Absorbance Measurement: Dilute the thiol dye conjugate to approximately 0.1 mg/mL and measure the absorbance at 280 nm (target molecule A₂₈₀) and at the dye's maximum absorbance wavelength (A_{max}).
- Correction Calculation: Calculate the corrected absorbance at 280 nm (A_{280C}) using the equation:
 - $A_{280C} = A_{280} - (A_{max} \times \text{Correction Factor})$

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