

Cyanine 5 bissuccinimidyl ester [equivalent to Cy5® bisNHS ester]

Catalog number: 157
Unit size: 1 mg

Component	Storage	Amount (Cat No. 157)
Cyanine 5 bissuccinimidyl ester [equivalent to Cy5® bisNHS ester]	Freeze (< -15 °C), Minimize light exposure	1 vial (1 mg)

OVERVIEW

A variety of cyanine 5 (Cy5®) dyes has been used to label biological molecules for fluorescence imaging and other fluorescence-based biochemical analysis. They are widely used for labeling peptides, proteins and oligos etc. Cy5® dyes are one type of the most common red fluorophores. These versatile fluorophores can tolerate a pH range of 3-10 for use in a variety of applications at biologically relevant pHs. The dyes are also DMSO tolerant and photostable to enable transfer from storage to assay without loss of performance. The aqueous solubility eliminates the need for organic solvents in the assay buffers. Our Cy5® Fluors are thoroughly QC tested to ensure high levels of chromophore and reactive dye content. Mono-reactive dyes are suitable for targeted, precise labeling of proteins and oligonucleotides and bis-reactive dyes are more suitable for general labeling. NHS ester dyes are recommended for labeling amine groups and maleimide dyes are recommended for labeling thiol groups. This Cy5® NHS ester readily reacts with amino groups. AAT Bioquest offers Cy dye NHS esters in the form of triethylammonium salts that are more soluble in DMSO and DMF than the corresponding potassium salts that are offered by some other vendors. The Cy dye triethylammonium salts have the same reactivity and give the conjugates identical to the the Cy dye potassium salts. Cy5® is the trademark of GE Healthcare.

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles

Protein stock solution (Solution A)

Mix 100 µL of a reaction buffer (e.g., 1 M sodium bicarbonate solution or 1 M phosphate buffer with pH ~9.0) with 900 µL of the target protein solution (e.g. antibody, protein concentration >2 mg/mL if possible) to give 1 mL protein labeling stock solution.

Note: The pH of the protein solution (Solution A) should be 8.5 ± 0.5. If the pH of the protein solution is lower than 8.0, adjust the pH to the range of 8.0-9.0 using 1 M sodium bicarbonate solution or 1 M pH 9.0 phosphate buffer.

Note: The protein should be dissolved in 1X phosphate buffered saline (PBS), pH 7.2-7.4. If the protein is dissolved in Tris or glycine buffer, it must be dialyzed against 1X PBS, pH 7.2-7.4, to remove free amines or ammonium salts (such as ammonium sulfate and ammonium acetate) that are widely used for protein precipitation.

Note: Impure antibodies or antibodies stabilized with bovine serum albumin (BSA) or gelatin will not be labeled well. The presence of sodium azide or thimerosal might also interfere with the conjugation reaction. Sodium azide or thimerosal can be removed by dialysis or spin column for optimal labeling results.

Note: The conjugation efficiency is significantly reduced if the protein concentration is less than 2 mg/mL. For optimal labeling efficiency the final protein concentration range of 2-10 mg/mL is recommended.

Cyanine 5 bissuccinimidyl ester stock solution (Solution B)

Add anhydrous DMSO into the vial of Cyanine 5 bissuccinimidyl ester

to make a 10 mM stock solution. Mix well by pipetting or vortex.

Note: Prepare the dye stock solution (Solution B) before starting the conjugation. Use promptly. Extended storage of the dye stock solution may reduce the dye activity. Solution B can be stored in freezer for two weeks when kept from light and moisture. Avoid freeze-thaw cycles.

SAMPLE EXPERIMENTAL PROTOCOL

This labeling protocol was developed for the conjugate of Goat anti-mouse IgG with Cyanine 5 bissuccinimidyl ester. You might need further optimization for your particular proteins.

Note: Each protein requires distinct dye/protein ratio, which also depends on the properties of dyes. Over labeling of a protein could detrimentally affects its binding affinity while the protein conjugates of low dye/protein ratio gives reduced sensitivity.

Run conjugation reaction

1. Use 10:1 molar ratio of Solution B (dye)/Solution A (protein) as the starting point: Add 5 µL of the dye stock solution (Solution B, assuming the dye stock solution is 10 mM) into the vial of the protein solution (95 µL of Solution A) with effective shaking. The concentration of the protein is ~0.05 mM assuming the protein concentration is 10 mg/mL and the molecular weight of the protein is ~200KD.

Note: We recommend to use 10:1 molar ratio of Solution B (dye)/Solution A (protein). If it is too less or too high, determine the optimal dye/protein ratio at 5:1, 15:1 and 20:1 respectively.

2. Continue to rotate or shake the reaction mixture at room temperature for 30-60 minutes.

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: For immediate use, the dye-protein conjugate need be diluted with staining buffer, and aliquoted for multiple uses.

Note: For longer term storage, dye-protein conjugate solution need be concentrated or freeze dried.

Characterize the Desired Dye-Protein Conjugate

Measure absorption

Read OD (absorbance) at 280 nm and dye maximum absorption (λ_{max} = 651 nm for Cyanine 5 bisuccinimidyl ester dyes)

Calculate DOS

[illegible]

Figure 1. Chemical structure for Cyanine 5 bissuccinimidyl ester [equivalent to Cy5® bisNHS ester]

DISCLAIMER

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