mFluor™ Blue 659 SE

Catalog number: 1181
Unit size: 1 mg

Component	Storage	Amount (Cat No. 1181)
mFluor™ Blue 659 SE	Freeze (< -15 °C), Minimize light exposure	1 mg

OVERVIEW

The mFluor™ Blue 659 dye is optimally excited with a 488 nm blue laser, offering a significant Stokes shift with an emission peak at approximately 659 nm. This water-soluble dye and its protein conjugates exhibit robust red fluorescence when excited at 488 nm, making them ideal for flow cytometry. Compared to RPE (R-Phycoerythrin), mFluor™ Blue 659 dyes are more photostable, enhancing their suitability for fluorescence imaging. Additionally, mFluor™ Blue 659 serves as a distinctive fluorochrome for spectral flow cytometry due to its unique spectral profile, a rarity among available dyes.

The succinimidyl ester (SE) of mFluor™ Blue 659 is a widely used reagent for the conjugation of this dye to proteins or antibodies. Succinimidyl esters react selectively and efficiently with primary amines (such as the side chains of lysine residues or aminosilane-coated surfaces) at pH 7-9, forming stable covalent amide bonds. This property makes mFluor™ Blue 659 SE an excellent choice for labeling proteins, amine-modified oligonucleotides, and other amine-containing molecules.

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)

1. Mix 100 µL of a reaction buffer (e.g., 1 M sodium carbonate 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. The final protein concentration range of 2-10 mg/mL is recommended for optimal labeling efficiency.

mFluor™ Blue 659 SE Stock Solution (Solution B)

1. Add anhydrous DMSO into the vial of mFluor™ Blue 659 SE to make a 10 mM stock solution. Mix well by pipetting or vortex.

   Note: Before starting the conjugation, prepare the dye stock solution (Solution B). Use promptly. Extended storage of the dye stock solution may reduce the dye activity. Solution B can be stored in the freezer for two weeks when protected 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 mFluor™ Blue 659 SE. 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 a 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 using a 10:1 molar ratio of Solution B (dye)/Solution A (protein). If it is too low 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 Conjugate

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

The Degree of Substitution (DOS) is the most important factor for characterizing dye-labeled protein. Proteins of lower DOS usually have weaker fluorescence intensity, but proteins of higher DOS (e.g., DOS > 6) tend to have reduced fluorescence too. The optimal DOS for most antibodies is recommended between 2 and 10, depending on the properties of dye and protein. For effective labeling, the degree of substitution should be controlled to have 6-8 moles of mFluor™ Blue 659 SE to one mole of antibody. The following steps are used to determine the DOS of mFluor™ Blue 659-labeled proteins.

Measure Absorption

To measure the absorption spectrum of a dye-protein conjugate, it is recommended to keep the sample concentration in the range of 1-10 μM depending on the extinction coefficient of the dye.

Read OD (absorbance) at 280 nm and dye maximum absorption (λmax = 503 nm for mFluor™ Blue 659 dyes

For most spectrophotometers, the sample (from the column fractions) needs to be diluted with de-ionized water so that the O.D. values are in the range of 0.1 to 0.9. The O.D. (absorbance) at 280 nm is the maximum absorption of protein, while 503 nm is the maximum absorption of mFluor™ Blue 659 SE. To obtain accurate DOS, ensure the conjugate is free of the non-conjugated dye.

Calculate DOS

You can calculate the DOS using our tool by following this link:

https://www.aatbio.com/tools/degree-of-labeling-calculator

EXAMPLE DATA ANALYSIS AND FIGURES

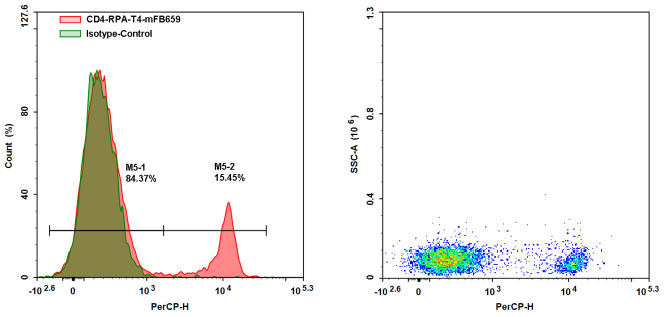

Figure 1. Flow cytometry analysis was performed on whole blood cells stained with mFluor™ Blue 659 anti-human CD4 antibody (Clone: RPA-T4). The fluorescence signal was detected using the NovoCyte 3000 flow cytometer in the PerCP channel.

DISCLAIMER

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