

## mFluor™ Blue 585 SE

Catalog number: 1159  
Unit size: 1 mg

Component	Storage	Amount (Cat No. 1159)
mFluor™ Blue 585 SE	Freeze (< -15 °C), Minimize light exposure	1 mg

### OVERVIEW

mFluor™ Blue 585 dyes are an excellent alternative to RPE since they have the spectral properties equivalent to those of RPE conjugates. mFluor™ Blue 585 dyes are water-soluble, and the protein conjugates prepared with mFluor™ Blue 585 dyes are well excited at 488 nm to give red fluorescence (compatible with TRITC filter). mFluor™ Blue 585 dye and conjugates are excellent blue laser reagents for flow cytometry applications. Compared to RPE, mFluor™ Blue 585 dyes are much more photostable, making them readily available for fluorescence imaging applications while it is very difficult to use RPE conjugates for fluorescence imaging applications due to the rapid photobleaching of RPE conjugates. mFluor™ Blue 585 are small organic dyes, thus they are much more readily conjugated to proteins with much higher yield than PE. AAT Bioquest's mFluor™ dye family provides the largest collection of fluorescent labeling dyes with large Stokes Shift. They are optimized for labeling proteins, in particular, antibodies. These dyes are bright, photostable and have minimal quenching on proteins. They have been validated for multicolor applications, in particular, for spectral flow cytometric applications. They can be well excited by the major laser lines of spectral flow cytometers (e.g., 350, 405, 488, 555, 633 and 647 nm).

### 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 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. For optimal labeling efficiency the final protein concentration range of 2-10 mg/mL is recommended.

#### mFluor™ Blue 585 SE stock solution (Solution B)

Add anhydrous DMSO into the vial of mFluor™ Blue 585 SE 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 mFluor™ Blue 585 SE. You might need further optimization for your particular proteins.

**Note:** Each protein requires a distinct dye/protein ratio, which also depends on the properties of dyes. Over-labeling of a protein could detrimentally affect its binding affinity while the protein conjugates of low dye/protein ratio give 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 conjugation

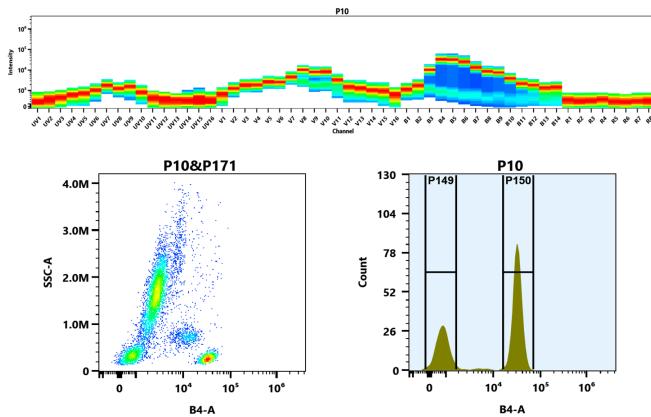
The following protocol is an example of dye-protein conjugate purification by using a Sephadex G-25 column.

1. Prepare Sephadex G-25 column according to the manufacturer 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 needs to be diluted with staining buffer, and aliquoted for multiple uses.

**Note:** For longer-term storage, the dye-protein conjugate solution needs to be concentrated or freeze-dried.

#### EXAMPLE DATA ANALYSIS AND FIGURES



**Figure 1.** Flow cytometry analysis of whole blood cells stained with mFluor™ Blue 585 anti-human CD4 antibody (Clone: SK3). The fluorescence signal was monitored using an Aurora flow cytometer in the mFluor™ Blue 585 specific B4-A channel.

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