mFluor™ Blue 620 SE

Catalog number: 1163
Unit size: 1 mg

Component	Storage	Amount (Cat No. 1163)
mFluor™ Blue 620 SE	Freeze (< -15 °C), Minimize light exposure	1 mg

OVERVIEW

mFluor™ Blue 620 dye can be well excited with blue laser at 488 nm. It has a huge Stokes shift with emission ~620 nm. mFluor™ Blue 620 dyes are water-soluble, and the protein conjugates prepared with mFluor™ Blue 620 dyes are well excited at 488 nm to give red fluorescence. mFluor™ Blue 620 dye and conjugates are excellent blue laser reagents for flow cytometry detections. Compared to RPE, mFluor™ Blue 620 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. It is also a unique fluorochrome for spectral flow cytometry since there are very few existing dyes that have this spectral profile.

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 it is lower than 8.0, adjust it to 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. For optimal labeling results, sodium azide or thimerosal can be removed by dialysis or spin column.

   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 620 SE stock solution (Solution B)

1. Add anhydrous DMSO into the vial of mFluor™ Blue 620 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 a 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 620 SE. You might need further optimization for your particular proteins.

Each protein requires a distinct dye/protein ratio, which also depends on the properties of the 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 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, dilute the dye-protein conjugate with staining buffer and aliquot for multiple uses.

   Note: For longer-term storage, the dye-protein conjugate solution must 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 proteins. Proteins with lower DOS usually have weaker fluorescence intensity, but proteins with higher DOS tend to have reduced fluorescence, too. The optimal DOS for most antibodies is between 2 and 10, depending on the properties of the dye and protein. The following steps are used to determine the DOS of mFluor™ Blue 620 SE 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 = 616 nm for mFluor™ Blue 620 dyes)

For most spectrophotometers, the sample (from the column fractions) need be diluted with de-ionized water so that the OD 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 616 nm is the maximum absorption of mFluor™ Blue 620 SE. To obtain accurate DOS, make sure that the conjugate is free of the non-conjugated dye.

Calculate DOS

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

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

EXAMPLE DATA ANALYSIS AND FIGURES

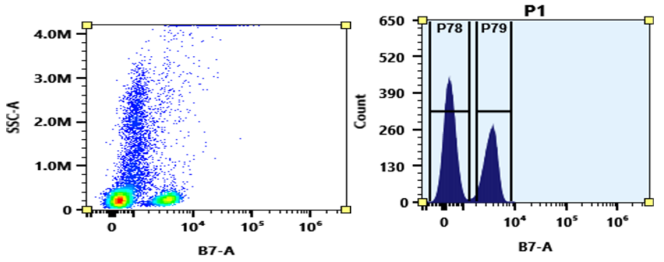

Figure 1. Flow cytometry analysis of whole blood stained with mFluor™ Blue 620 anti-human CD4 *SK3* conjugate. The fluorescence signal was monitored using an Aurora spectral flow cytometer in the mFluor™ Blue 620 specific B7-A channel.

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