ReadiLink™ Rapid mFluor™ Violet 420 Antibody Labeling Kit *Microscale Optimized for Labeling 50 µg Antibody Per Reaction*

Catalog number: 1105
Unit size: 2 Labelings

Component	Storage	Amount (Cat No. 1105)
Component A: mFluor™ Violet 420	Freeze (< -15 °C), Minimize light exposure	2 vials (One vial is for 50 µg protein)
Component B: Reaction Buffer	Freeze (< -15 °C), Minimize light exposure	1 vial (20 µL)
Component C: TQ™-Dyed Quench Buffer	Freeze (< -15 °C), Minimize light exposure	1 vial (20 µL)

OVERVIEW

AAT Bioquest's mFluor™ dyes are developed for flow cytometry-focused applications. These dyes can be well excited by the laser lines of flow cytometers (e.g., 405 nm, 488 nm and 633 nm). mFluor™ Violet 420 dyes have fluorescence excitation and emission maxima of ~405 nm and ~420 nm respectively. These spectral characteristics make them an excellent replacement for Cascade® Blue labeling dye (Cascade® Blue is the trademark of InvitroGen). mFluor™ Violet 420 SE is reasonably stable and shows good reactivity and selectivity with protein amino groups. This kit has all the essential components for labeling ~100 µg antibody with no column purification needed. mFluor™ Violet 420 SE antibody labeling kit provides a convenient method to label monoclonal, polyclonal antibodies or other proteins (>10 kDa) with the Violet Laser-excitable mFluor™ Violet 420 SE.

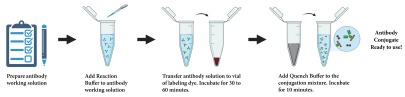

Figure 1. Overview of the ReadiLink™ Rapid Antibody Labeling protocol. In just two simple steps, and with no purification necessary, covalently label microgram amounts of antibodies in under an hour.

AT A GLANCE

Important

Warm all the components and centrifuge the vials briefly before opening them. Immediately prepare the necessary solutions before starting your conjugation. The following protocol is a recommendation.

PREPARATION OF WORKING SOLUTION

Protein working solution (Solution A)

For labeling 50 µg of protein (assuming the target protein concentration is 1 mg/mL), mix 5 µL (10% of the total reaction volume) of Reaction Buffer (Component B) with 50 µL of the target protein solution.

Note: If you have a different protein concentration, adjust the protein volume accordingly to make ~50 µg of protein available for your labeling reaction.

Note: For labeling 100 µg of protein (assuming the target protein concentration is 1 mg/mL), mix 10 µL (10% of the total reaction volume) of Reaction Buffer (Component B) with 100 µL of the target protein solution.

Note: The protein should be dissolved in 1X phosphate buffered saline (PBS), pH 7.2 - 7.4; if the protein is dissolved in glycine buffer, it must be dialyzed against 1X PBS, pH 7.2 - 7.4, or use Amicon Ultra-0.5, Ultracel-10 Membrane, 10 kDa (cat# UFC501008 from Millipore) 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.

Note: For optimal labeling efficiency, a final protein concentration range of 1 - 2 mg/mL is recommended, with a significantly reduced conjugation efficiency at less than 1 mg/mL.

SAMPLE EXPERIMENTAL PROTOCOL

Run conjugation reaction

1. Add the protein working solution (Solution A) to ONE vial of labeling dye (Component A), and mix them well by repeatedly pipetting for a few times or vortex the vial for a few seconds.

   Note: If labeling 100 µg of protein, use both vials (Component A) of labeling dye by dividing the 100 µg of protein into 2 x 50 µg of protein and reacting each 50 µg of protein with one vial of labeling dye. Then combine both vials for the next step.

2. Keep the conjugation reaction mixture at room temperature for 30 - 60 minutes.

   Note: The conjugation reaction mixture can be rotated or shaken for a longer time if desired.

Stop Conjugation reaction

1. Add 5 µL (for 50 µg protein) or 10 µL (for 100 µg protein) which is 10% of the total reaction volume of TQ-Dyed Quench Buffer (Component C) into the conjugation reaction mixture; mix well.
2. Incubate at room temperature for 10 minutes. The labeled protein (antibody) is now ready to use.

Storage of Protein Conjugate

The protein conjugate should be stored at > 0.5 mg/mL in the presence of a carrier protein (e.g., 0.1% bovine serum albumin). For longer storage, the protein conjugates could be lyophilized or divided into single-used aliquots and stored at ≤ –20°C.

EXAMPLE DATA ANALYSIS AND FIGURES

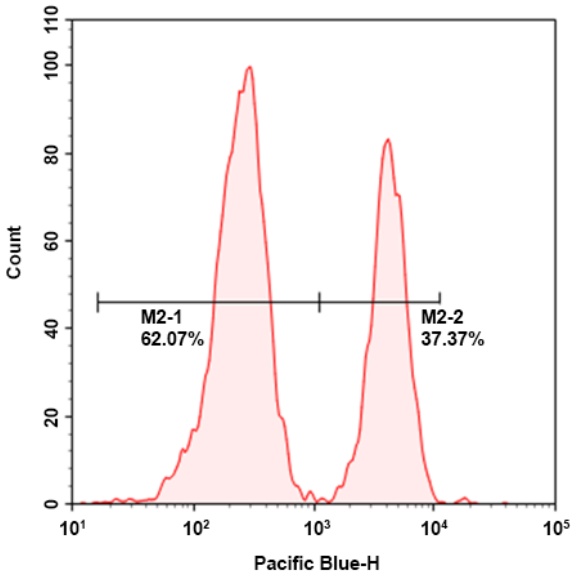

Figure 1. Detection of CD4 expression on human peripheral blood lymphocytes stained by flow cytometry. Human PBMCs were stained with mFluor™ Violet 420 anti-human CD4 monoclonal antibody *SK3*. The fluorescence signal was monitored using an ACEA NovoCyte flow cytometer in the Pacific Blue channel.

DISCLAIMER

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