ReadiLink™ Rapid mFluor™ Violet 610 Antibody Labeling Kit *Microscale Optimized for Labeling 50 µg Antibody Per Reaction*

Catalog number: 1116
Unit size: 2 Labelings

Component	Storage	Amount (Cat No. 1116)
Component A: mFluor™ Violet 610	Freeze (< -15 °C), Minimize light exposure	2 vials (1 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

ReadiLink™ Rapid mFluor™ Violet 610 Antibody Labeling Kits provide a quick and convenient method to label antibodies, proteins (>10 kDa), or other amine-containing biomolecules. Leveraging a unique conjugation chemistry, ReadiLink™ Antibody Labeling Kits enables researchers to label microscale volumes of antibodies in two easy steps, with no purification needed and 100% recovery of the labeled product. Each kit includes sufficient mFluor™ Violet 610 dye to facilitate two distinct labeling reactions of 50 µg antibody samples. The resulting conjugates are ideal for a wide range of applications, including flow cytometry, fluorescent microscopy techniques, immunocytochemistry (ICC), immunohistochemistry (IHC), ELISA, and indirect FISH. Compared to Alexa Fluor dyes and conventional dye counterparts, mFluor™ dyes are brighter, more photostable, and resistance to changes in pH between pH 4 to 10. The mFluor™ Violet 610 dyes are optimally excited by violet lasers and emit red fluorescence ~610 nm. These properties make them well-suited for spectral fluorescence flow cytometry, providing researchers with a tool for detailed biological analysis with unparalleled precision and sensitivity. With ReadiLink™ Rapid Antibody Labeling kits, researchers can directly label primary antibodies, eliminating the need for secondary antibodies and enhancing panel-building flexibility.

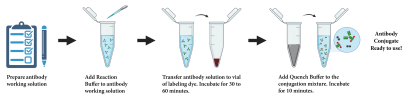

Figure 1. Overview of the ReadiLink™ Rapid Antibody Labeling protocol. In just two simple steps, and with no purification necessary, covalently label microgram amounts of antibodies in under an hour.

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)

1. 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 the ReadiUse™ 10KD Spin Filter (Cat. 60502) 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: A final protein concentration range of 1 - 2 mg/mL is recommended for optimal labeling efficiency, 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 vortexing 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 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

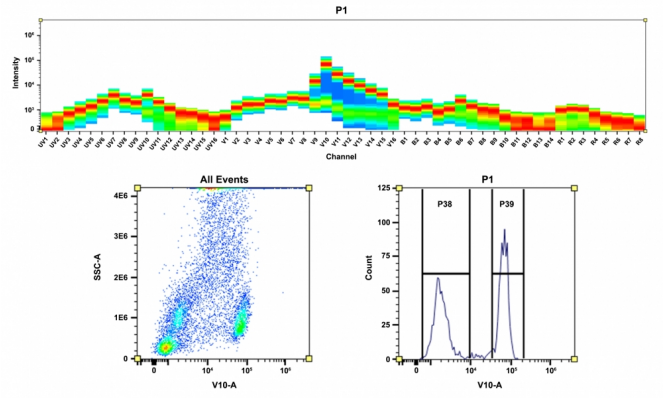

Figure 1. Top) Spectral pattern was generated using a 4-laser spectral cytometer. Spatially offset lasers (355 nm, 405 nm, 488 nm, and 640 nm) were used to generate four distinct emission profiles, then, when combined, yielded the overall spectral signature. Bottom) Flow cytometry analysis of whole blood cells stained with CD4-mFluor™ Violet 610 conjugate. Conjugates were prepared using the ReadiLink™ Rapid mFluor™ Violet 610 Antibody Labeling Kit. The fluorescence signal was monitored using a Cytek® Aurora spectral flow cytometer in the mFluor™ Violet 610 specific V10-A channel.

DISCLAIMER

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