ReadiLink™ Rapid mFluor™ Violet 510 Antibody Labeling Kit *Microscale Optimized for Labeling 50 µg Antibody Per Reaction*

Catalog number: 1110
Unit size: 2 Labelings

Component	Storage	Amount (Cat No. 1110)
Component A: mFluor™ Violet 510	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 have large Stokes Shifts, and can be well excited by the laser lines of flow cytometers (e.g., 405 nm, 488 nm and 633 nm). mFluor™ Violet 510 dyes have fluorescence excitation and emission maxima of ~405 nm and ~510 nm respectively. mFluor™ Violet 510 SE is reasonably stable and shows good reactivity and selectivity with protein amino groups. This kit has all the essential components to perform two separate labeling reactions with no column purification needed. Each of the two vials of mFluor™ Violet 510 SE provided in the kit is optimized for labeling ~50 µg antibody. mFluor™ Violet 510 SE antibody labeling kit provides a convenient method to label small amounts of monoclonal, polyclonal antibodies or other proteins (>10 kDa) with the Violet Laser-excitable mFluor™ Violet 510 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

Before starting your conjugation, it is recommended to warm all the components and briefly centrifuge the vials. Prepare the necessary solutions immediately upon opening the vials.

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 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: 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 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 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 the TQ-Dyed Quench Buffer (Component C) into the conjugation reaction mixture, and 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

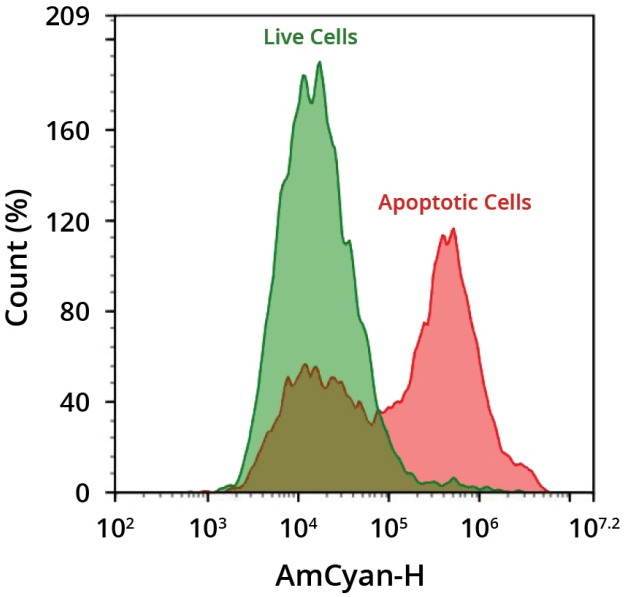

Figure 1. Flow cytometric analysis of cells undergoing apoptosis using Annexin V-mFluor™ Violet 510. Jurkat cells were treated with (red) or without 1 µM staurosporine (green) for 4 hours at 37 °C. Cells were then incubated with Annexin V labeled using the ReadiLink™ Rapid mFluor™ Violet 510 Antibody Labeling Kit (Cat No. 1110) for 30 minutes to identify apoptotic cells. Fluorescence intensity was measured using an ACEA NovoCyte flow cytometer.

DISCLAIMER

AAT Bioquest provides high-quality reagents and materials for research use only. For proper handling of potentially hazardous chemicals, please consult the Safety Data Sheet (SDS) provided for the product. Chemical analysis and/or reverse engineering of any kit or its components is strictly prohibited without written permission from AAT Bioquest. Please call 408-733-1055 or email info@aatbio.com if you have any questions.