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
Component A: mFluor™ Violet 420	Freeze (<-15 °C), Minimize light exposure	2 vials (One vial is for 50 µg protein)
Component B: Reaction Buffer	Refrigerate (2-8 °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.

AT A GLANCE

Table 1. Available fluorophores in AAT Bioquest ReadyLink™ Rapid Antibody Labelling Kits

Cat#	Labels	Ex (nm)	Em (nm)
1100	mFluor™ Violet 450	403	454
1105	mFluor™ Violet 420	398	411
1110	mFluor™ Violet 510	414	508
1114	mFluor™ Violet 540	399	550
1120	mFluor™ Blue 570	553	570
1123	mFluor™ Green 620	522	617
1126	mFluor™ Yellow 630	561	630
1130	mFluor™ Red 700	657	700
1131	mFluor™ Red 780	629	780
1220	iFluor™ 350	345	442
1227	iFluor™ 555	559	569
1230	iFluor™ 594	592	614
1235	iFluor™ 647	654	674
1240	iFluor™ 680	682	701
1245	iFluor™ 700	693	713
1250	iFluor™ 750	753	779
1255	iFluor™ 488	491	514
1260	iFluor™ 633	638	655
1265	iFluor™ 790	782	811
1290	Cy3	555	565
1292	Cy5	644	665
1294	Cy7	749	776
1299	FITC	494	520

PREPARATION OF WORKING SOLUTION

Important

Warm all the components and centrifuge the vials briefly before opening, and immediately prepare the required solutions before starting your conjugation. The following protocol is for recommendation.

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 longer time if desired.

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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

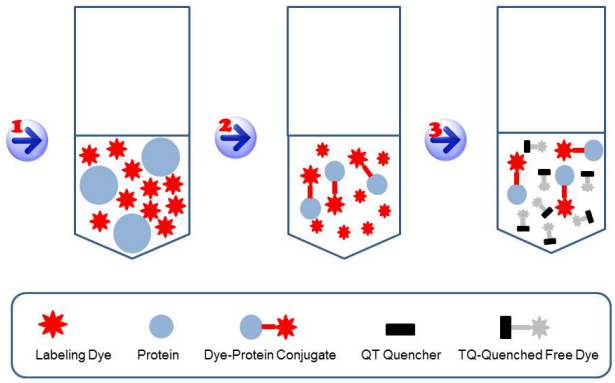

Figure 1. Readilink™ Kit Labeling Principle: 1). Start the labeling reaction by mixing a labeling dye with a protein (to be labeled) in the Reaction Buffer (pH 7.5-8.5). 2). Incubation gives a mixture of the desired protein conjugate and unreactive free dye. 3). Quench the reaction by mixing a non-fluorescent Tide Quencher™ (TQ) dye with the reaction solution. The TQ dye stops the reaction AND converts the unreactive free labeling dye to the non-fluorescent TQ-Labeling dye complex, which eliminates the background fluorescence interference of the free labeling dye.

DISCLAIMER