

**ReadiLink™ Rapid mFluor™ Violet 450  
Antibody Labeling Kit \*Microscale  
Optimized for Labeling 50 µg Antibody Per  
Reaction\***

Catalog number: 1100

Unit size: 2 Labelings

| Component                           | Storage                                       | Amount                                  |
|-------------------------------------|---|---|
| Component A: mFluor™ Violet 450     | 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 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 450 dyes have fluorescence excitation and emission maxima of ~405 nm and ~450 nm respectively. These spectral characteristics make them an excellent replacement for Pacific Blue™ labeling dye. mFluor™ Violet 450 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 450 SE provided in the kit is optimized for labeling ~50 µg antibody. mFluor™ Violet 450 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 450 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.

### Stop Conjugation reaction

1. Add 5  $\mu\text{L}$  (for 50  $\mu\text{g}$  protein) or 10  $\mu\text{L}$  (for 100  $\mu\text{g}$  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  $\leq -20^{\circ}\text{C}$ .

### EXAMPLE DATA ANALYSIS AND FIGURES

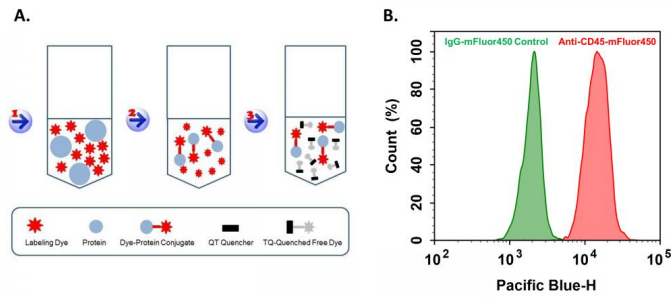


Figure 1.

Expression of CD45 in differentiated HL-60 cells were quantified using labeled anti-CD45 antibody by ReadiLink™ Rapid mFluor™ Violet 450 Antibody Labeling Kit (Cat# 1100). **A.** ReadiLink™ Kit Labeling Principle. **B.** HL-60 cells were with 1.25% DMSO for 4 days to differentiate. The live cells were incubated with 1  $\mu\text{g}/\text{ml}$  anti-CD45-mFluor450 or IgG-mFluor450 control and analyzed by NovoCyte.

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