ReadiLink™ Rapid mFluor™ Yellow 630 Antibody Labeling Kit *Microscale Optimized for Labeling 50 µg Antibody Per Reaction*

Catalog number: 1126
Unit size: 2 Labelings

Component	Storage	Amount
Component A: mFluor™ Yellow 630	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 . mFluor™ Yellow 630 dyes have fluorescence excitation and emission maxima of ~561 nm and ~630 nm respectively, making its conjugates readily excitable by 561 nm yellow laser. These spectral characteristics make mFluor™ Yellow 630 an excellent choice to make yellow laser-excitable antibodies for flow cytometry applications. mFluor™ Yellow 630 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 and purifications. Each of the two vials of mFluor™ Yellow 630 provided in the kit is optimized for labeling ~50 µg antibody. mFluor™ Yellow 630 antibody labeling kit provides a convenient method to label small amounts of monoclonal, polyclonal antibodies or other proteins (>10 kDa) with the mFluor™ Yellow 630 SE dye.

AT A GLANCE

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.

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 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

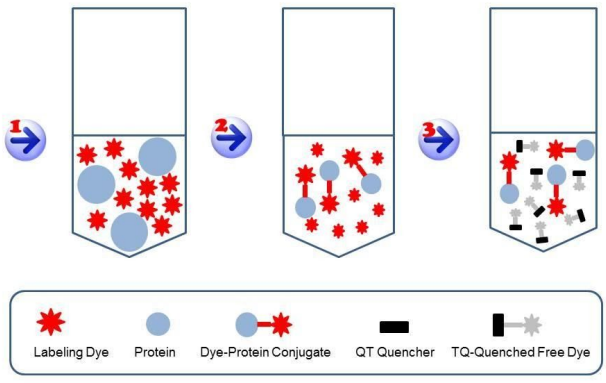

Figure 1. Readilink™ Kit Labeling Principle: 1). the labeling reaction by mixing a labeling dye with a protein (to be labeled) in the Reaction Buffer (pH 7.5-8.5). 2). gives a mixture of the desired protein conjugate and unreactive

free dye. 3). 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

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.