ReadiLink™ Rapid mFluor™ Red 780 Antibody Labeling Kit *Microscale Optimized for Labeling 50 µg Antibody Per Reaction*

Catalog number: 1131
Unit size: 2 Labelings

Component	Storage	Amount (Cat No. 1131)
Component A: mFluor™ Red 780	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™ Red 780 dyes have fluorescence excitation and emission maxima of ~629 nm and ~780 nm respectively. Like Alexa Fluor 750-APC tandem, it has strong absorption at 633 nm, making its conjugates readily excitable by 633 nm red laser. These spectral characteristics make mFluor™ Red 780 an excellent alternative to Alexa Fluor 700-APC tandem. mFluor™ Red 780 is a small organic molecule that is much easier to use than the Alexa Fluor 750-APC tandem. mFluor™ Red 780 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™ Red 780 provided in the kit is optimized for labeling ~50 µg antibody. mFluor™ Red 780 antibody labeling kit provides a convenient method to label small amounts of monoclonal, polyclonal antibodies or other proteins (>10 kDa) with the mFluor™ Red 780 dye.

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)

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 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 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 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

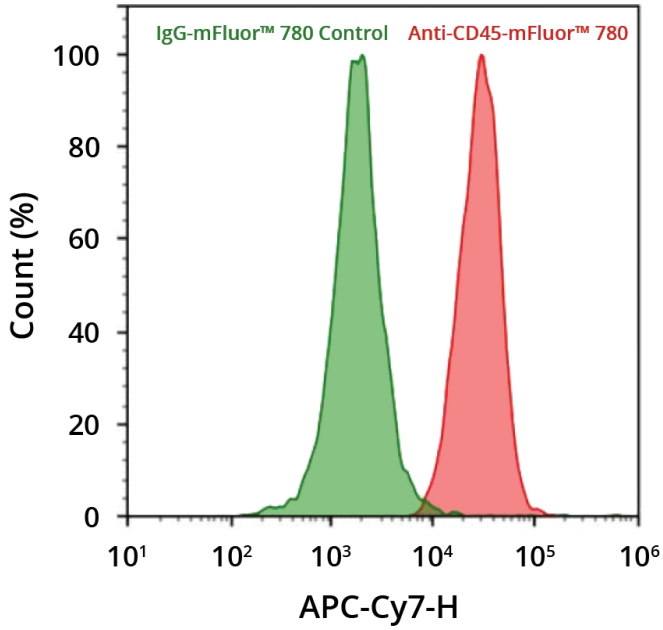

Figure 1. Flow cytometric analysis of CD45 expression in differentiated HL-60 cells quantified using an anti-CD45 antibody labeled using the ReadiLink™ Rapid mFluor™ Red 780 Antibody Labeling Kit (Cat No. 1131). HL-60 cells were treated with 1.25% DMSO for 4 days to differentiate. Live cells were then incubated with 1 µg/mL anti-CD45-mFluor™ 780 or IgG-mFluor™ 780 control and analyzed by NovoCyte.

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.