ReadiLink™ Rapid iFluor® 750 Antibody Labeling Kit *Microscale Optimized for Labeling 50 µg Antibody Per Reaction*

Catalog number: 1250
Unit size: 2 Labelings

Component	Storage	Amount (Cat No. 1250)
Component A: iFluor® 750	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 iFluor® dyes are developed for labeling proteins, in particular, antibodies. These dyes are optimized to have minimal fluorescence quenching effect on proteins and nucleic acids. Our iFluor® 750 dyes have fluorescence excitation and emission maxima close to 750 nm and 780 nm respectively with good photostability. Our in-house comparable studies indicated that our iFluor® 750 dyes are significantly brighter than the corresponding Cy7® and Alexa Fluor® 750. These spectral characteristics make them a superior alternative to Cy7® and Alexa Fluor® 750 (Cy7® and Alexa Fluor® are the trademarks of GE Healthcare and Invitrogen respectively). iFluor® 750 conjugates have been widely used in fluorescence animal imaging applications. ReadiLink™ labeling kits essentially only require 2 simple mixing steps without a column purification needed. iFluor® 750 SE used in this ReadiLink™ kit is reasonably stable and shows good reactivity and selectivity with protein amino groups. The kit has all the essential components for labeling ~2x50 ug antibody. Each of the two vials of iFluor® 750 dye provided in the kit is optimized for labeling ~50 µg antibody. iFluor® 750 SE protein labeling kit provides a convenient method to label monoclonal, polyclonal antibodies or other proteins (>10 kDa) with the iFluor® 750 SE.

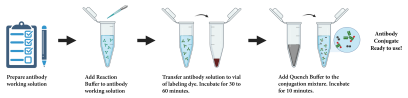

Figure 1. Overview of the ReadiLink™ Rapid Antibody Labeling protocol. In just two simple steps, and with no purification necessary, covalently label microgram amounts of antibodies in under an hour.

AT A GLANCE

Important

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

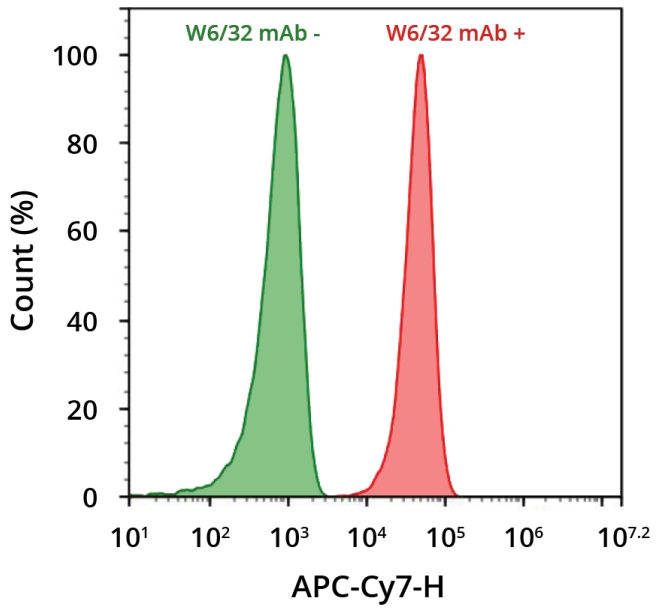

Figure 1. HL-60 cells were incubated with (red) or without (green) anti-human HLA-ABC (W6/32 mAb). Cells were then incubated with goat anti-mouse IgG labeled using the ReadiLink™ Rapid iFluor® 750 Antibody Labeling Kit (Cat No. 1250). The fluorescence signal was monitored using ACEA NovoCyte flow cytometer in the APC-Cy7 channel.

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.