ReadiLink™ xtra Rapid XFD750 Antibody Labeling Kit *BSA-Compatible*

Catalog number: 1988
Unit size: 2 Labelings

Component	Storage	Amount (Cat No. 1988)
Component A: Preactivated XFD750	Freeze (< -15 °C), Minimize light exposure	2 vials
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

XFD750 is manufactured by AAT Bioquest, and it has a chemical structure similar to that of Alexa Fluor® 750 (Alexa Fluor® is the trademark of Thermo Fisher). ReadiLink™ xtra rapid antibody labeling kits require essentially only 2 simple mixing steps without a column purification needed. Specially formulated and preactivated XFD750 (chemically equivalent to Alexa Fluor® 750) used in this ReadiLink™ kit is quite stable and shows good reactivity and selectivity with antibodies. The kit has all the essential components for labeling ~2x50 ug antibody. Each of the two vials of preactivated XFD750 dye provided in the kit is optimized for labeling ~50 µg antibody. ReadiLink™ xtra XFD750 rapid antibody labeling kit provides a convenient and robust method to label monoclonal and polyclonal antibodies with NIR fluorescent XFD750 fluorophore. XFD750 is one of the most used fluorophores for labeling antibodies.

AT A GLANCE

Important Note

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) with 0.1 to 0.5 % will 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

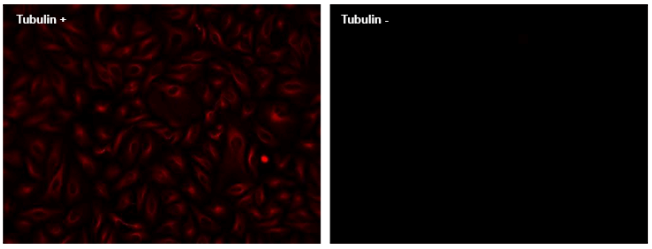

Figure 1. HeLa cells were incubated with (Tubulin+) or without (Tubulin-) mouse anti-tubulin followed by XFD750 goat anti-mouse IgG conjugate (H+L).

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.