ReadiLink™ xtra Rapid iFluor™ 594 Antibody Labeling Kit *BSA-Compatible*

Catalog number: 1960
Unit size: 2 Labelings

Component	Storage	Amount
Component A: Preactivated iFluor™ 594	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

ReadiLink™ xtra rapid antibody labeling kits require essentially only 2 simple mixing steps without a column purification needed. Preactivated iFluor™ 594 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 iFluor™ 594 dye provided in the kit is optimized for labeling ~50 µg antibody. ReadiLink™ xtra iFluor™ 594 rapid antibody labeling kit provides a convenient and robust method to label monoclonal and polyclonal antibodies with the bright red fluorescent iFluor™ 594 fluorophore. AAT Bioquest's iFluor™ dyes are optimized for labeling proteins, in particular, antibodies. These dyes are bright, photostable and have minimal quenching on proteins. They can be well excited by the major laser lines of fluorescence instruments (e.g., 350, 405, 488, 555 and 633 nm).

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) with 0.1 to 0.5 % will 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

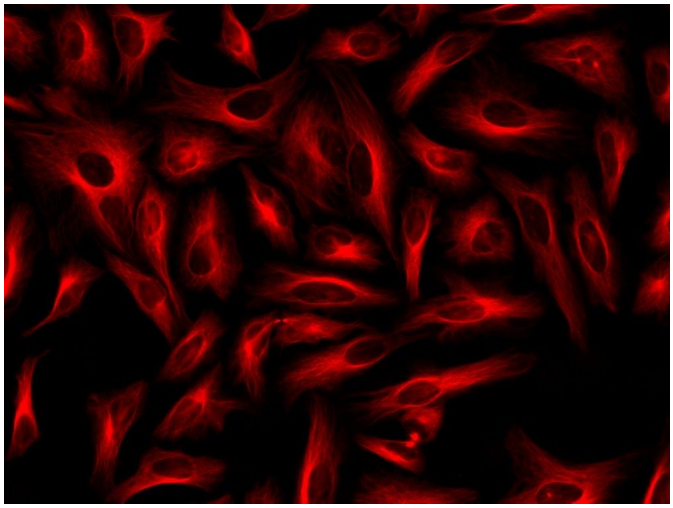

Figure 1. Immunofluorescence staining of tubulin in HeLa cells. HeLa cells were fixed with 4% PFA, permeabilized with 0.1% Triton X-100 and blocked. Cells were then incubated with mouse anti-tubulin monoclonal antibody and stained with a goat anti-mouse IgG labeled using the ReadiLink™ xtra Rapid iFluor™ 594 Antibody Labeling Kit (Cat No. 1960).

DISCLAIMER

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