

Xtra Rapid iFluor647 Antibody Labeling Kit (BSA-Compatible)

Cat#: orb867215 (Protocol)

Component Storage Amount

Component A: Preactivated iFluor™ 647 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

xtra rapid antibody labeling kits require essentially only 2 simple mixing steps without a column purification needed. Preactivated iFluor™ 647 used in this kit is quite stable and shows good reactivity and selectivity with antibodies. The kit has all the essential components for labeling ~2x50 µg antibody. Each of the two vials of preactivated iFluor™ 647 dye provided in the kit is optimized for labeling ~50 µg antibody. Xtra iFluor™ 647 rapid antibody labeling kit provides a convenient and robust method to label monoclonal and polyclonal antibodies with the bright red fluorescent iFluor™ 647 fluorophore. Biorbyt'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 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 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

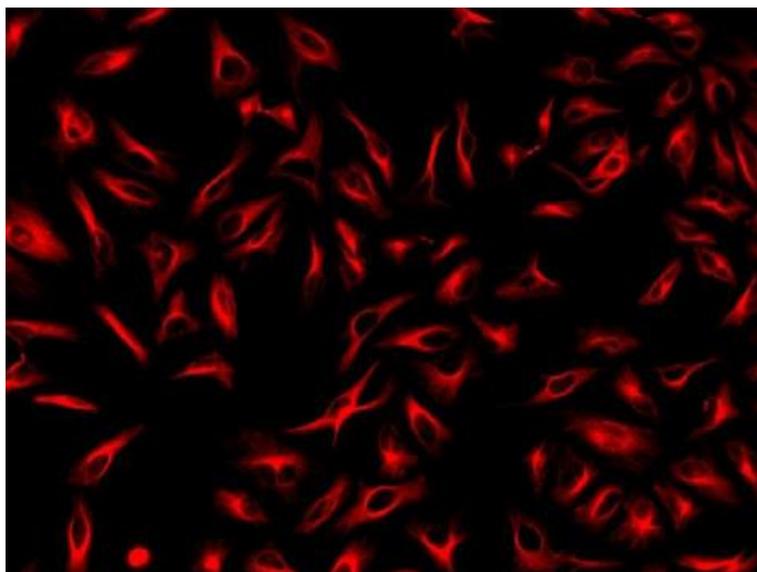


Figure 1. Immunofluorescence staining of tubulin in HeLa cells. HeLa cells were fixed with 4% PFA, permeabilized with 0.1% Triton X-100 and blocked. Cells were then incubated with mouse anti-tubulin monoclonal antibody and stained with a goat anti-mouse IgG labeled using the xtra Rapid iFluor™ 647 Antibody Labeling Kit.