ReadiLink™ Rapid iFluor® 405 Antibody Labeling Kit *Microscale Optimized for Labeling 50 µg Antibody Per Reaction*

Catalog number: 1228
Unit size: 2 Labelings

Component	Storage	Amount (Cat No. 1228)
Component A: iFluor® 405	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

ReadiLink™ Rapid iFluor® 405 Antibody Labeling Kit is a microscale optimized system designed for labeling antibodies with iFluor® 405, a violet-excitable fluorescent dye with excitation and emission maxima near 403 nm and 427 nm. This dye is ideal for use with the 405 nm laser line and is compatible with commonly used violet channels in flow cytometry and fluorescence imaging. The kit includes buffers and pre-aliquoted dye to support two separate labeling reactions, each optimized for 50 µg of antibody. The labeling process consists of two simple mixing steps and does not require column purification. This format supports consistent preparation of iFluor® 405-labeled antibodies for use in fluorescence-based detection workflows.

AT A GLANCE

1. Prepare solution A by mixing 5 µL Reaction Buffer (Component B) with 50 µL antibody in 1X PBS (pH 7.2–7.4) to label 50 µg antibody.
2. Add solution A to one vial of Labeling Dye (Component A), mix well, and incubate for 30–60 minutes at room temperature.
3. Add 5 µL TQ™-Dyed Quench Buffer (Component C) to the reaction, mix well, incubate for 10 minutes at room temperature, and use the labeled antibody directly.

Important Note

Warm all components to room temperature, briefly centrifuge the vials before opening, and prepare the required solutions immediately prior to starting the conjugation. This protocol is provided as a general 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: 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

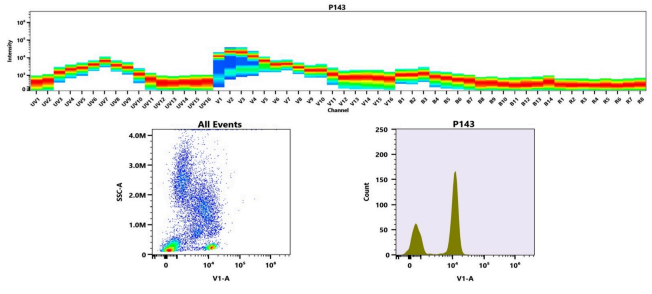

Figure 1. (Top) Spectral emission profiles generated using four

spatially offset lasers (355 nm, 405 nm, 488 nm, and 640 nm). Each laser produced a distinct emission pattern, and their combination yielded the composite spectral signature. (Bottom) Flow cytometry analysis of human whole blood stained with CD4 (RPA-T4) antibody labeled using ReadiLink™ Rapid iFluor® 405 Antibody Labeling Kit (Cat. #1228). The fluorescence signal was monitored on a Cytek Aurora spectral flow cytometer in the V1-A channel, demonstrating clear detection of $CD4^+$ lymphocytes.

DISCLAIMER

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