ReadiLink™ Rapid iFluor™ 680 Antibody Labeling Kit *Microscale Optimized for Labeling 50 µg Antibody Per Reaction*

Catalog number: 1240
Unit size: 2 Labelings

Component	Storage	Amount
Component A: iFluor™ 680	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 optimized for labeling proteins, in particular, antibodies. These dyes are optimized to have minimal fluorescence quenching effect on proteins and nucleic acids. Our iFluor™ 680 dyes have fluorescence excitation and emission maxima close to 680 nm and 700 nm respectively with good photostability. These spectral characteristics make them an excellent alternative to Cy5.5®, IRDye® 700 and Alexa Fluor® 680 (Cy5.5®, IRDye® and Alexa Fluor® are the trademarks of GE Healthcare, Li-COR and Invitrogen respectively). ReadiLink™ labeling kits essentially only require 2 simple mixing steps without a column purification needed. iFluor™ 680 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™ 680 dye provided in the kit is optimized for labeling ~50 µg antibody. iFluor™ 680 SE protein labeling kit provides a convenient method to label monoclonal, polyclonal antibodies or other proteins (>10 kDa) with the iFluor™ 680 SE.

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) 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

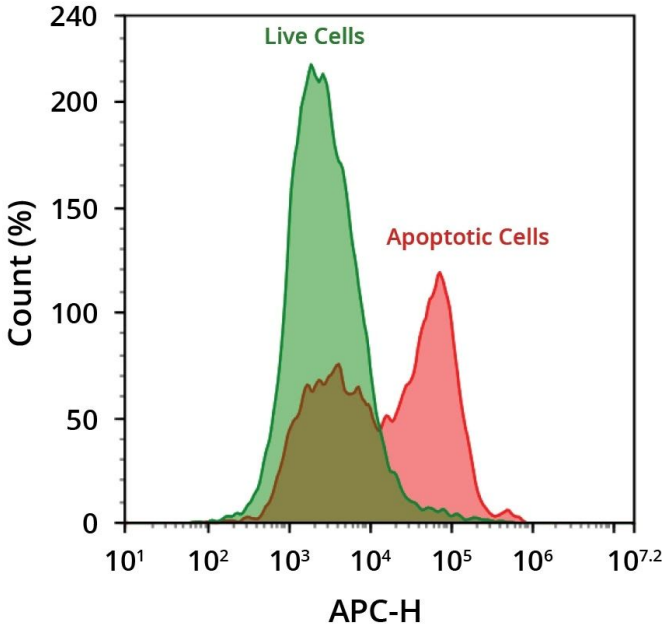

Figure 1. Flow cytometric analysis of cells undergoing apoptosis using Annexin V-iFluor™ 680. Jurkat cells were treated with (red) or without 1 µM staurosporine (green) for 4 hours at 37 ºC. Cells were then incubated with Annexin V labeled using the ReadiLink™ Rapid iFluor™ 680 Antibody Labeling Kit (Cat No. 1240) for 30 minutes to identify apoptotic cells. Fluorescence intensity was measured using an ACEA NovoCyte flow cytometer.

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.