ReadiLink™ iFluor™ 647 Protein Labeling Kit

Microscale Optimized for Labeling ~100 µg Antibody Per Reaction

Ordering Information	*Storage Conditions*
Product Number: 1235 (2 reactions)	Multiple storage conditions required

Introduction

iFluor™ 647 dyes have fluorescence excitation and emission maxima close to 647 nm and 660 nm respectively. These spectral characteristics make them an excellent alternative to Cy5® and Alexa Fluor® 647 labeling dye (Cy5® and Alexa Fluor® are the trademarks of GE Healthcare and Invitrogen respectively). iFluor™ 647 SE used in the kit is reasonably stable and shows good reactivity and selectivity with protein amino groups. The iFluor™ 647 dye has also been used in microarray applications in combination with iFluor™ 555. It demonstrated much higher photostability. Our kit is optimized for labeling about 100 µg antibodies. It provides a convenient method to label small amount of monoclonal, polyclonal antibodies or other proteins (>10 kDa).

Kit Components

Components	Amount	Storage
Component A: iFluor™ 647 SE	1 vial	-20 °C
Component B: Reaction Buffer	1 vial (200 µL)	Do not freeze
Component C: DMSO	1 vial (100 µL)	-20 °C
Component D: Spin Column	2 columns	Do not freeze
Component E: Washing Tube (2 mL)	2 tubes	Do not freeze
Component F: Collecting Tube (1.5 mL)	2 tubes	Do not freeze

Storage and Handling

Upon receipt, store iFluor™ 647 SE (Component A) at -20 °C, kept from light and moisture. Store other components at room temperature. When stored properly, the kit components should be stable for six months.

Note: Do not freeze Reaction Buffer (Component B) and Spin Column (Component D).

Standard Operating Protocol

Warm all the components before opening, and immediately prepare the required solutions before starting the conjugation. Avoid repeated freeze thaw cycles if possible. The dye-containing solutions should be kept from light. You might need further optimization for your protein labeling since this SOP was developed for labeling anti-CD4 antibody.

1. Prepare protein solution (Solution A):

Assuming the concentration of the target protein solution (antibody solution) is 2 mg/mL, mix 5 µL (10% of the total reaction volume) of Reaction Buffer (Component B) with 50 µL of the target protein solution. If you have a difference protein concentration, adjust the protein volume accordingly to make ~100 µg protein available for this labeling reaction**.**

Note 1*: The pH of the protein solution (Solution A) should be 8.5 ± 0.5. If the pH of the protein solution is lower than 8.0, adjust the pH to the range of 8.0-9.0 using Reaction Buffer (Component B) or saturated sodium bicarbonate solution.*

Note 2*: The protein should be dissolved in 1X phosphate buffered saline (PBS), pH 7.2-7.4; If the protein is dissolved in Tris or glycine buffer, it must be dialyzed against 1X PBS, pH 7.2-7.4, to remove free amines or ammonium salts (such as ammonium sulfate and ammonium acetate) that are widely used for protein precipitation.*

Note 3*: Impure antibodies or antibodies stabilized with bovine serum albumin (BSA) or gelatin will not be labeled well. The presence of sodium azide or thimerosal might also interfere with the conjugation reaction. Sodium azide or thimerosal can be removed by dialysis or spin column for optimal labeling results.*

Note 4*: The conjugation efficiency is significantly reduced if the protein concentration is less than 2 mg/mL. For optimal labeling efficiency the final protein concentration range of 2-10 mg/mL is recommended.*

2. Prepare dye stock solution (Solution B):

Add 10µL of DMSO (Component C) into the vial of iFluor™ 647 SE (Component A), and vortex the vial vigorously.

Note 1: *Prepare the dye stock solution (Solution B) before starting the conjugation. Use promptly. Extended storage of the dye stock solution may reduce the dye activity. Solution B can be stored in freezer for two weeks when kept from light and moisture.*

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Note 2: *Aliquot the dye stock solution into 5 vials (2μL/vial). ONLY one vial is needed for labeling 100 ug proteins. The remaining vials can be stored in freezer for 2 weeks.*

3. Run conjugation reaction:

3.1 Add the protein solution (Solution A) to the vial of dye stock solution (2 μL/vial, Solution B), and mix them well by repeatedly pipetting for 2-5 minutes.

3.2 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.*

4. Prepare spin column for sample purification:

4.1 Invert the Spin Column (Component D) several times to resuspend the settled gel and remove any bubbles.

4.2 Snap off the tip and place the column in the Washing Tube (2 mL, Component E). Remove the cap to allow the excess packing buffer to drain by gravity to the top of the gel bed. If column does not begin to flow, push cap back into column and remove it again to start the flow. Discard the drained buffer, and then place the column back into the Washing Tube. However, centrifuge immediately if the column is placed into a 12 x 75 mm test tube (not provided).

4.3 Centrifuge for 1 min in a swinging bucket centrifuge at 1,000 x g (see Centrifugation Notes section) to remove the packing buffer. Discard the buffer.

4.4 Apply 2 mL 1X PBS (pH 7.2-7.4) to the column. After each application of PBS, let the buffer drain out by gravity, or centrifuge the column for 1 min to remove the buffer. Discard the buffer from the collection tube. Repeat this process for 3-4 times.

4.5 Centrifuge for 2 minutes in a swinging bucket centrifuge at 1,000 x g (see Centrifugation Notes section) to remove the packing buffer. Discard the buffer.

5. Purify the conjugation:

5.1 Place the column (from Step 4.5) in a clean Collecting Tube (1.5 mL, Component F) or 12 x 75 mm test tube. Carefully load the sample (20–100 μL) directly to the center of the column.

5.2 After loading the sample, add 1X PBS (pH 7.2-7.4) to make the total volume of 110 μL. Centrifuge the column for 5 min at 1,000 x g, and collect the solution that contains the desired dye-labeled protein.
Note 1: *For immediate use, the dye-protein conjugate need be diluted with staining buffer, and aliquoted for multiple uses.*
Note 2: *For longer term storage, dye-protein conjugate solution need be concentrated or freeze dried (see below).*

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). The conjugate solution could be stored at 4 °C for two months without significant change when stored in the presence of 2 mM sodium azide and kept from light. For longer storage, the protein conjugates could be lyophilized or divided into single-used aliquots and stored at ≤ –60 °C. PROTECT FROM LIGHT.

Centrifugation Notes

Spin Column (Component D) can fit into 2 mL microcentrifuge tubes or 12 x 75 mm test tubes for sample collection during centrifugation. Use the 2 mL microtubes provided with the columns for the initial column equilibration step.

Swinging bucket centrifuges capable of generating a minimum force of 1,000 x g are suitable for Bio-Spin column use. The gravitational force created at a particular revolution speed is a function of the radius of the microcentrifuge rotor. Consult the swinging bucket centrifuge instruction manual for the information about conversion from revolutions per minute (RPM) to centrifugal or g-force. Alternatively, use the following equation to calculate the speed in RPM required to reach the gravitational force of 1,000 x g.
RCF (x g) = $(1.12 \times 10^{-5}) \times (\text{RPM}) \times 2 \times r$ (*RCF is the relative centrifugal force, r is the radius in centimeters measured from the center of the rotor to the middle of the Bio-Spin column, and RPM is the speed of the rotor*).

References

1. Hermanson GT (1996). *Biocojugate Techniques*, Academic Press, New York.
2. Haugland RP (1995). Coupling of monoclonal antibodies with fluorophores. *Methods Mol Biol* **45,** 205-21.
3. Brinkley M (1992). A brief survey of methods for preparing protein conjugates with dyes, haptens, and cross-linking reagents. *Bioconjugate Chem* **3,** 2-13.