

ReadiLink™ trFluor™ Tb and trFluor™ Eu Protein Labeling Kits

Microscale Optimized for Labeling 50-100 µg Antibody Per Reaction

Cat. #	Product Name	Label dyes	Ex (nm)	Em (nm)
1300	ReadiLink™ trFluor™ Eu Protein Labeling Kit	trFluor™ Eu	346	617
1305	ReadiLink™ trFluor™ Tb Protein Labeling Kit	trFluor™ Tb	330	544

Introduction

Many biological compounds present in cells, serum or other biological fluids are naturally fluorescent, and thus the use of conventional, prompt fluorophores leads to serious limitations in assay sensitivity due to the high background caused by the autofluorescence of the biological molecules to be assayed. The use of long-lived fluorophores combined with time-resolved detection (a delay between excitation and emission detection) minimizes prompt fluorescence interferences. AAT Bioquest's ReadiLink™ trFluor™ Tb and trFluor™ Eu protein labeling kits provide one of the most convenient ways to label proteins in microscale. Our trFluor™ Tb and trFluor™ Eu probes enable time-resolved fluorometry (TRF) for the assays that require high sensitivity. These trFluor™ probes have large Stokes shifts and extremely long emission half-lives when compared to more traditional fluorophores such as Alexa Fluor or cyanine dyes. Compared to the other TRF compounds, our trFluor™ Eu and trFluor™ Tb probes have relatively high stability, high emission yield and ability to be linked to biomolecules. Moreover, our trFluor™ Eu and trFluor™ Tb probes are insensitive to fluorescence quenching when conjugated to biological polymers such as antibodies. Each ReadiLink™ protein labeling kit provides all the essential components for performing two conjugation reactions (for 2x50µg protein). 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: Labeling Dye	2 vials (One vial is for 50 µg protein)	-20 °C
Component B: Reaction Buffer	1 vial (20 µL)	Do not freeze
Component C: Spin Column	2 columns	Do not freeze
Component D: Washing Tube (2 mL)	2 tubes	Do not freeze
Component E: Collecting Tube (1.5 mL)	2 tubes	Do not freeze

Standard Operating Protocol (Labeling 50 µg Protein)

Upon receipt, store Labeling dye (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 1: Do not freeze Reaction Buffer (Component B) and Spin Column (Component C).

Note 2: Warm all the components and centrifuge the vials briefly before opening, and immediately prepare the required solutions before starting your conjugation. The following SOP is an example for labeling anti-HDAC IgG antibody

1. Prepare protein solution (Solution A):

For labeling 50 µg protein (assuming the target protein concentration is 1 mg/mL), mix 1.5 µL (3% of the total reaction volume) of Reaction Buffer (Component B) with 50 µL of the target protein solution.

Note 1: If you have a difference protein concentration, adjust the protein volume accordingly to make ~50 µg protein available for your labeling reaction.

Note 2: For labeling 100 µg protein (assuming the target protein concentration is 1 mg/mL), mix 3 µL (3% of the total reaction volume) of Reaction Buffer (Component B) with 100 µL of the target protein solution.

Note 3: 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 4: Impure antibodies or antibodies stabilized with bovine serum albumin (BSA) or gelatin will not be labeled well.

Note 5: The conjugation efficiency is significantly reduced if the protein concentration is less than 1 mg/mL. For optimal labeling efficiency the final protein concentration range of 1-2 mg/mL is recommended.

2. Run conjugation reaction:

- 2.1 Add the protein 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: Use both vials (Component A) of labeling dye to label 100 µg protein by dividing the 100 µg protein into 2x50 µg protein and reacting each 50 µg protein with one vial of labeling dye. Combine two vials for the next step.

- 2.2 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.

3. Prepare spin column for sample purification:

- 3.1 Invert the Spin Column (Component C) several times to resuspend the settled gel and remove any bubbles.
- 3.2 Snap off the tip and place the column in the Washing Tube (2 mL, Component D). 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).
- 3.3 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.
- 3.4 Apply 1-2 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.
- 3.5 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.

4. Purify the conjugation:

- 4.1 Place the column (from Step 3.5) in a clean Collecting Tube (1.5 mL, Component E). Carefully load the sample (50–100 µL) directly to the center of the column.
- 4.2 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 ≤ -20 °C. PROTECT FROM LIGHT.

Centrifugation Notes

Spin Column (Component C) 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. $RCF (x\ g) = (1.12 \times 10^{-5}) \times (RPM)^2 \times r$ (*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

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