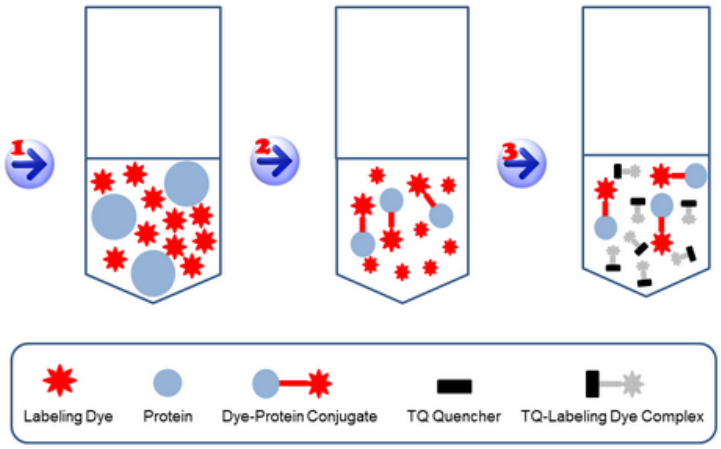
ReadiLink™ Antibody Labeling Kits

Microscale Optimized for Labeling 50-100 µg Antibody Per Reaction

Introduction

ReadiLink™ antibody labeling kits provide one of the most convenient ways to label antibodies in microscale. The kits only require two simple mixing steps without a purification step involved. The succinimidyl esters (SE) of *i*Fluor™ and mFluor™ dyes used in the kits show good reactivity and selectivity with the aliphatic amines of proteins and forms a carboxamide bond, which is identical to, and is as stable as the natural peptide bond. The *i*Fluor™and mFluor-antibody conjugates may be used for immuno-fluorescent staining, fluorescence *in situ* hybridization, flow cytometry and other biological applications. Each ReadiLink™ antibody labeling kit provides all the essential components for performing two conjugation reactions (for 2x50µg protein). Each kit can be used to label 50-100 µg monoclonal, polyclonal antibodies or other proteins (>10 kDa) with only two simple mixing steps.



Readilink™ Kit Labeling Principle

1. ***Start*** the labeling reaction by mixing a labeling dye with a protein (to be labeled) in the Reaction Buffer (pH 7.5-8.5).
2. ***Incubation*** gives a mixture of the desired protein conjugate and unreactive free dye.
3. ***Quench*** the reaction by mixing a non-fluorescent Tide Quencher™ (TQ) dye with the reaction solution. The TQ dye stops the reaction AND converts the unreactive free labeling dye to the non-fluorescent TQ-Labeling dye complex, which eliminates the background fluorescence interference of the free labeling dye.

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)	-20 °C
Component C: TQ™-Dyed Quench Buffer	1 vial (20 µL)	-20 °C

Standard Operating Protocol (Labeling 50 µg Protein)

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 5 µL (10% 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 10 µL (10% 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.*

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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. Stop Conjugation reaction:

3.1 Add 5 µL (for 50 µg protein*)* or 10µL (for 100 µg protein) which is 10% of the total reaction volume of TQ-Dyed Quench Buffer (Component C) into the conjugation reaction mixture (from step 2.2), mix them well.

3.2 Incubate at room temperature for 10 minutes.

3.3 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.

Table 1. AAT Bioquest ReadiLink™ Antibody Labeling Kits (2 Labelings/Kit, Each Labeling is for 50 µg Antibody)

Cat. #	Product Name	Labels	Ex (nm)	Em (nm)
1299	ReadiLink™ FITC Antibody Labeling Kit	FITC	494	520
1220	ReadiLink™ *i*Fluor™ 350 Antibody Labeling Kit	*i*Fluor™ 350	345	442
1255	ReadiLink™ *i*Fluor™ 488 Antibody Labeling Kit	*i*Fluor™ 488	491	514
1227	ReadiLink™ *i*Fluor™ 555 Antibody Labeling Kit	*i*Fluor™ 555	559	569
1230	ReadiLink™ *i*Fluor™ 594 Antibody Labeling Kit	*i*Fluor™ 594	592	614
1260	ReadiLink™ *i*Fluor™ 633Antibody Labeling Kit	*i*Fluor™ 633	638	655
1235	ReadiLink™ *i*Fluor™ 647 Antibody Labeling Kit	*i*Fluor™ 647	654	674
1240	ReadiLink™ *i*Fluor™ 680 Antibody Labeling Kit	*i*Fluor™ 680	682	701
1245	ReadiLink™ *i*Fluor™ 700 Antibody Labeling Kit	*i*Fluor™ 700	693	713
1250	ReadiLink™ *i*Fluor™ 750 Antibody Labeling Kit	*i*Fluor™ 750	753	779
1265	ReadiLink™ *i*Fluor™ 790 Antibody Labeling Kit	*i*Fluor™ 790	782	811
1105	ReadiLink™ *m*Fluor™ Violet 420 Antibody Labeling Kit	*m*Fluor™ Violet 420	398	411
1100	ReadiLink™ *m*Fluor™ Violet 450 Antibody Labeling Kit	*m*Fluor™ Violet 450	403	454
1110	ReadiLink™ *m*Fluor™ Violet 510 Antibody Labeling Kit	*m*Fluor™ Violet 510	414	508
1114	ReadiLink™ *m*Fluor™ Violet 540 Antibody Labeling Kit	*m*Fluor™ Violet 540	399	550
1120	ReadiLink™ mFluor™ Blue 570 Antibody Labeling Kit	mFluor™ Blue 570	553	570
1123	ReadiLink™ mFluor™ Green 620 Antibody Labeling Kit	mFluor™ Green 620	522	617
1126	ReadiLink™ mFluor™ Yellow 630 Antibody Labeling Kit	mFluor™ Yellow 630	561	630
1130	ReadiLink™ mFluor™ Red 700 Antibody Labeling Kit	mFluor™ Red 700	657	700
1131	ReadiLink™ mFluor™ Red 780 Antibody Labeling Kit	mFluor™ Red 780	629	780

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.