ReadiLink™ Rapid XFD750 Antibody Labeling Kit *Production Scale*

Catalog number: 5745
Unit size: 3 x 1 mg

Component	Storage	Amount (Cat No. 5745)
Component A: XFD750	Freeze (< -15 °C), Minimize light exposure	3 Vials (Lyophilized Powder)
Component B: Reaction Buffer	Freeze (< -15 °C), Minimize light exposure	1 Vial (200 µL)
Component C: DMSO	Refrigerated (2-8 °C)	1 Vial (100 µL)
Component D: Spin Column	Refrigerated (2-8 °C)	3 Columns

OVERVIEW

ReadiLink™ Rapid Antibody Labeling Kits, designed for production scale, provide a convenient and efficient method for labeling large volumes of antibodies with our superior iFluor® dyes, XFD dyes (equivalent to Alexa Fluor®), and various other labels. These kits utilize reactive fluorophores modified with succinimidyl ester (SE) functional groups, which selectively bind to primary amines on proteins, resulting in remarkably bright and photostable conjugates. Every kit contains all the necessary components for three distinct labeling reactions and features a user-friendly, pre-packed spin column for efficient dye removal, maximizing conjugate yield. Each vial of XFD750 dye provided in the kit is precisely formulated to label 1 mg of purified protein or antibody. Before labeling, it is important to remove stabilizing proteins like BSA from the sample and refrain from using amine-rich buffers like Tris, which might disrupt the labeling process. The dye XFD750 is a bright, near-NIR fluorescent dye with an excitation and emission maxima of ~752 nm and ~776 nm, respectively. The structure of XFD750 is similar to that of Alexa Fluor® 750, making XFD750 conjugates an excellent alternative for imaging and flow cytometry applications. With ReadiLink™ Rapid Antibody Labeling kits, researchers can directly label primary antibodies, eliminating the need for secondary antibodies and enhancing panel-building flexibility.

AT A GLANCE

Key Parameters for Optimal Results

1. 1.0 mg Antibody (MW ~150 kDa)
2. Antibody concentration: 2.0 mg/mL
3. Antibody volume: 500 µL

SAMPLE EXPERIMENTAL PROTOCOL

Important

Before opening the vials, warm all components and briefly centrifuge. Immediately prepare necessary solutions before starting conjugation. This protocol is a recommendation.

Antibody Labeling Reaction

1. Warm up a vial of reactive dye (Component A) to room temperature.

   Note: Each vial of reactive dye contains an optimized amount of dye to label 1 mg of IgG (MW ~150 kDa) at 2 mg/mL in PBS, the kit can also be used to label other proteins (>10 kDa).

2. Add 10 µL of DMSO (Component D) to the vial of reactive dye (Component A), mix well.

3. Prepare a 500 µL antibody solution in PBS with a concentration of 2 mg/mL.

   Note: The protein should be dissolved in 1X phosphate buffered saline (PBS), pH 7.2 - 7.4. If the protein is dissolved in buffers containing primary amines, like Tris and/or glycine, it must be dialyzed against 1X PBS, pH 7.2 - 7.4, or use Amicon Ultra0.5, Ultracel-10 Membrane, 10 kDa (Cat No. 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.

4. Add 25 µL of Reaction Buffer (Component B) to the antibody solution.

5. Transfer the reconstituted dye solution into the vial of antibody solution, and pipette several times to mix well.

6. Rotate the reaction mixture for 1 hour at room temperature.

Purification with Desalting Column

1. Twist off the bottom closure of the desalting column (Component D), and loosen the cap. Place the column in a collection tube.

2. Centrifuge the column at 1,000 g for 2 minutes to remove the storage solution.

3. Remove the cap and slowly add 1 mL of PBS to the column. Centrifuge at 1,000 g for 2 minutes and remove the buffer. Repeat this step 3 additional times, discarding the buffer from the collection tube each time.

4. Place the column in a new collection tube, and gently apply the sample into the center of the compact resin bed.

5. Centrifuge the column at 1,000 g for 2 minutes to collect the sample.

Determine the Antibody Concentration & Degree of Labeling (Optional)

The following formula can be used to calculate the antibody concentration:

$$(A_{280} - CF_{280} \times A_{dye}) / 1.4$$

The following formula can be used to calculate the degree of labeling:

$$DOL = (A_{dye} / Ec_{dye}) / (A_{280} - CF_{280} \times A_{dye}) / 210{,}000)$$

Where:

- 210,000 is the molar extinction coefficient (Ec) in $cm^{-1}M^{-1}$ of IgG at 280 nm.
- CF_{280} is the correction factor for the effect of the fluorophore on absorbance at 280 nm.
- A_{dye} is the absorbance at maximum (λ_{max}) for the respective dye.

Table 1. Properties of Labeling Dyes found in the ReadiLink™ Rapid Antibody Labeling Kits.

Cat#	Dye	Mol. Wt.	Ec ($cm^{-1}M^{-1}$)	CF_{280}	Target DOL
5700	iFluor® 350	749.85	20,000	0.23	5-10
5702	iFluor® 488	945.07	75,000	0.21	4-8
5705	iFluor® 555	914.06	90,000	0.16	4-7
5710	iFluor® 594	1160.42	18,000	0.04	3-6
5713	iFluor® 647	1274.66	250,000	0.03	3-7
5718	iFluor® 750	1416.83	250,000	0.039	2-6
5720	FITC	620.52	75,000	0.183	3-6
5722	Cy3	829.03	150,000	0.073	1-3
5725	Cy5	855.07	250,000	0.03	2-4
5727	Cy7	881.11	250,000	0.036	2-4
5730	XFD488	643.4	71,000	0.11	4-8
5733	XFD555	1250	150,000	0.08	4-7
5736	XFD594	819.85	90,000	0.56	3-6
5740	XFD647	1259.66	240,000	0.03	3-7
5745	XFD750	1300	240,000	0.04	2-5

EXAMPLE DATA ANALYSIS AND FIGURES

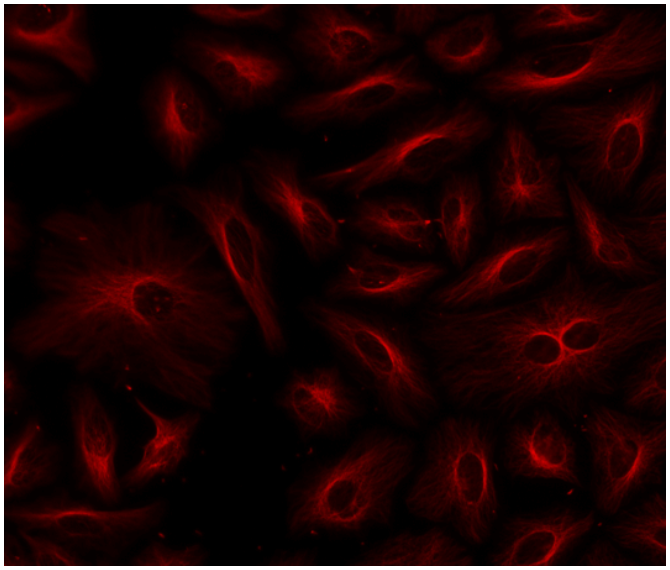

Figure 1. HeLa cells were labeled with mouse anti-tubulin followed by a goat anti-mouse IgG conjugated to XFD750 using the ReadiLink™ Rapid XFD750 Antibody Labeling Kit (Cat No. 5745).

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