ab312813 – XFD750 Conjugation Kit (BSA compatible)

For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab312813

Materials Supplied

Item	Quantity	Storage Condition
Preactivated XFD750	2 vials	-20°C
Reaction Buffer	1 vial	-20°C
TQ™-Dyed Quench Buffer	1 vial	-20°C

Reagent Preparation

Warm all the components and centrifuge the vials briefly before opening them. Immediately prepare the necessary solutions before starting your conjugation. The following protocol is a recommendation.

Protein working solution (Preactivated XFD750): 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 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 with 100 µL of the target protein solution.

Note: The antibody should be dissolved in 1X phosphate buffered saline (PBS), pH 7.2-7.4; If the antibody is dissolved in glycine buffer, it must be dialyzed against 1X PBS, pH 7.2-7.4, or use a 10KD Spin Filter (Antibody Concentration And Clean-Up Kit (ab102778)) to remove free amines or ammonium salts (such as ammonium sulfate and ammonium acetate) that are widely used for antibody precipitation.

Note: Impure antibodies or antibodies stabilized with bovine serum albumin (BSA) with 0.1 to 0.5 % will be labelled well. Higher concentrations of BSA will affect conjugation efficiency and should be either diluted or purified.

Note: A final protein concentration range of 1 - 2 mg/mL is recommended for optimal labeling efficiency, with a significantly reduced conjugation efficiency at less than 1 mg/mL.

Assay Procedure

Run conjugation reaction

1. Add the protein working solution (Preactivated XFD750) to ONE vial of labeling dye, 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 (Preactivated XFD750) 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 a 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 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.

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