# ab312804 – mFluor™ Violet 450 Conjugation Kit

For research use only - not intended for diagnostic use.

**For overview, typical data and additional information please visit: www.abcam.com/ab312804**

## Materials Supplied

| Item | Quantity | Storage Condition |
|---|---|---|
| mFluor™ Violet 450 | 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 (mFluor™ Violet 450):** 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 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**: Impure antibodies or antibodies stabilized with bovine serum albumin (BSA) or gelatin will not be labeled well. For BSA removal, we recommend BSA Removal Kit (ab173231). For purification and concentration we recommend Antibody Concentration And Clean-Up Kit (ab102778).

**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 (mFluor™ Violet 450) 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 (mFluor™ Violet 450) 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.


Version 1b | 2023-09-07