Alkyne Protein Labeling Kit (1mg scale)

A1510883

Storage: Room Temperature. 2-8℃. -20℃. Protect from light. For detailed storage information, please refer to the kit contents.

Shipping: Shipped with ice packs. Please store under the specified storage conditions immediately upon receipt.

SKU	Prod. Name	Size
A1510882	Alkyne Protein Labeling Kit (100 μg scale)	10 reactions
A1510883	Alkyne Protein Labeling Kit (1mg scale)	10 reactions
A1510884	Alkyne Protein Labeling Kit (5mg scale)	10 reactions

Introduction

Aladdin's Alkyne Protein Labeling Kit (1mg scale) (A1510883) enables the rapid and efficient introduction of alkyne groups into target proteins containing primary amine groups, allowing subsequent labeling via click chemistry. Proteins and antibodies labeled with this kit can be quickly and efficiently conjugated to azide-tagged biomolecules under copper(I) catalysis. The resulting conjugates are suitable for labeling, tracking, purification, or isolation, and can be used in studies of protein–protein, protein–biomolecule, and DNA–protein interactions, making it an important tool in medical and life science research.

The kit includes reagents for Alkyne-labeling and purification of ten 500μL samples with a concentration of 1-10 mg/mL. Labeling and purification can be performed in as little as 90 minutes. The kit is optimized for labeling proteins with molecular weights between 20 kDa and 150 kDa.

Kit Contents

A1510883	Component	Appearance	10 reactions	Storage	Quantity Per Reaction
A1510883A	Alkyne-NHS	Light yellow viscous liquid	5mg	-20℃. Store in the dark.	Prepare according to instructions
A1510883B	DMSO	Colorless clear liquid	1mL	RT	Prepare according to instructions
A1510883C	Purification Resin	White bead slurry	30mL	2-8℃. Do not freeze	3mL for 1 reaction
A1510883D	Empty Spin Column	Transparent tub	10EA	RT.	1EA for 1 reaction

Required materials not supplied

1. Centrifuge.
2. 2mL microcentrifuge tubes.
3. Desired Protein for labeling (free of BSA or any carrier protein).
4. PBS buffer (pH 7.2-7.4).
5. UV-Vis spectrophotometer.

Matters needing attention

1. The purified proteins should be in a buffer that does not contain primary amines (for example, azide, ammonium ions, Tris, glycine, ethanolamine, triethylamine, glutathione) or imidazole. All of these substances significantly inhibit protein labeling.
2. Impure proteins or proteins stabilized with bovine serum albumin (BSA) or gelatin will not be labeled well.
3. The crosslinker is moisture-sensitive. Equilibrate the vial to room temperature before opening to prevent moisture condensation inside the vial. After preparing the stock solution with the anhydrous solvent provided in the kit, it can be aliquoted and stored at –20 °C, where it remains stable for two months. Storage at –80 °C allows for even longer preservation.
4. The sample loading volume for Spin Desalting Columns must be between 400 and 700 µL to ensure optimal performance. Loading volumes outside this range may lead to lower protein recovery or inefficient removal of unconjugated reagents.
5. Do not reuse Spin Desalting Columns.

Instruction for use

1. Prepare the protein sample

1.1 Prepare the target protein in a buffer (pH 7-9) free of amines, ammonium ions, or azide as these will interfere with the labeling reaction. If necessary, dialyze or exchange the target protein into an appropriate buffer, such as PBS (1×), before labeling.

1.2 Prepare the protein sample at a concentration of 1-10 mg/mL in PBS solution. The final volume should be ≤700 µL.

2. Calculations

The optimal amount of Alkyne-NHS will depend on the desired degree of labeling. In general, higher protein concentrations or proteins with a greater number of accessible primary amines (e.g., lysine residues) on the surface will require less Alkyne-NHS to achieve the desired labeling density. For recommendations on Alkyne:protein ratios, see Table 1.

Table 1. Recommended molar ratio of Alkyne-NHS to target protein

Protein concentration Range	Molar Ratio (Alkyne:Protein)
1.0-5.0 mg/mL	50:1-20:1
5.0-10 mg/mL	20:1-10:1

2.1 Use the following formula to calculate the amount (in millimoles) of crosslinker to add to the sample for an X:1 molar ratio.

mmol Alkyne-NHS = (mg/mL protein × mL protein)/ MW protein(mg/mmol) × molar ratio

2.2 Use the following formula to calculate the volume (in µL) of 10 mg/mL crosslinker to add to the sample.

µL Alkyne-NHS = (mmol Alkyne-NHS × 225.2 / 10) × 1000

2.3 For 500 µL of 2 mg/mL Streptavidin (MW = 60,000 g/mol) with 20:1 molar ratio, add 7.5 µL of 10 mg/mL Alkyne-NHS to the prepared sample.

mmol Alkyne -NHS = (2mg/mL protein × 0.5mL protein) / 60000 (mg/mmol) × 20 = 3.3×10^{-4} mmol

µL Alkyne-NHS = (3.3×10^{-4} mmol Alkyne-NHS × 225.2 / 10) × 1000 = 7.5 µL

3 Protein labeling procedure

3.1 Equilibrate Alkyne-NHS ester to room temperature.

3.2 Just before use, reconstitute the crosslinker. Add 500 µL of DMSO to the vial of Alkyne-NHS to obtain a 10 mg/mL stock solution. Vortex or pipette up and down until the solution is homogeneous. If not to be used immediately, dispense into aliquots and store at –20°C.

3.3 Add the calculated volume of 10 mg/mL Alkyne-NHS to the tube.

3.4 Incubate the reaction mixture for 1 hour at room temperature or 2 hours on ice.

4 Prepare the spin column

4.1 Place an empty spin column in a 15mL collection tube.

4.2 Suspend the purification resin by repeated inversion of the reagent bottle. Then add 3.0 mL of the suspension into the column and allow the resin to settle. Allow the column buffer to drain from the column by gravity. Initially, some pressure may be required to cause the first few drops of buffer to elute. Centrifuge the column at 1,000 × g for 2 minutes, discard the storage buffer and return column to the same collection tube.

Note: The purification resin is supplied as a slurry in 20% ethanol, with a resin-to-ethanol volume ratio of 3:1 (v/v).

4.3 Equilibrate the column by adding 2mL of PBS to the top of the resin bed and centrifuging at 1,000 × g for 2 minutes. Discard the flowthrough and repeat this step a total of 3 times.

Note: When using a fixed-angle rotor, place a mark on the side of the column that faces away from the rotor center. For all subsequent centrifugation steps, place the column in the microcentrifuge with the mark facing away from the rotor center.

5 Purify the Alkyne-labeled protein

5.1 Transfer the equilibrated column into a new collection tube.

5.2 Carefully pipette the entire reaction mixture (≤700 µL) onto the center of the column.

5.3 Centrifuge the column-tube assembly at 1,000×g for 2 minutes. The purified Alkyne-labeled protein is in the collection tube.