Alkyne Protein Labeling Kit (100 μg scale)

A1510882

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 (100 μg scale) (A1510882) 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 100μL 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

A1510882	Component	Appearance	10 reactions	Storage	Quantity Per Reaction
A1510882A	Alkyne-NHS	Light yellow viscous liquid	2mg	-20℃. Store in the dark.	Prepare according to instructions
A1510882B	DMSO	Colorless clear liquid	1mL	RT	Prepare according to instructions
A1510882C	Spin Desalting Column	Column	10 EA	2-8℃. Do not freeze	1 EA for 1 reaction
A1510882D	Collection Tubes	Transparent tub	10 EA	RT.	1 EA for 1 reaction

Required materials not supplied

1. Microcentrifuge.
2. 1.5mL 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 70 and 120 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 ≤120 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 100 µL of 2 mg/mL Streptavidin (MW = 60,000 g/mol) with 20:1 molar ratio, add 1.5 µL of 10 mg/mL Alkyne-NHS to the prepared sample.

mmol Alkyne -NHS = (2mg/mL protein × 0.1mL protein) / 60000 (mg/mmol) × 20 = 6.7×10^{-5} mmol

µL Alkyne-NHS = (6.7×10^{-5} mmol Alkyne-NHS × 225.2 / 10) × 1000 = 1.5 µL

3 Protein labeling procedure

3.1 Equilibrate Alkyne-NHS ester to room temperature.

3.2 Just before use, reconstitute the crosslinker. Add 200 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 Loosen the cap on a spin column, twist the tab off of the bottom, then place the column into a collection tube.

4.2 Centrifuge the column-tube assembly at 1,000×g for 2 minutes to remove the storage solution.

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.

4.3 Discard the flowthrough, then place the column back into the collection tube.

4.4 Add 500 µL of PBS Buffer, then centrifuge the column-tube assembly at 1,000×g for 2 minutes to equilibrate the column. Discard the flow through.

4.5 Repeat step 4.4 3 times, discarding the buffer from the collection tube each time.

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 (≤120 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.