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Life science solutions

# Protein Biotinylation Kit manual

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The **Protein Biotinylation Kit** is a ready-to-use kit for covalent labeling of purified proteins, peptides, and antibodies with biotin (3–5 biotin molecules per protein) using an activated biotin NHS ester. The kit enables rapid, reproducible biotinylation of primary amine groups (ε-amino groups of lysine residues and N-terminal amino groups) without significantly affecting the protein's biological activity.

The kit contains components for performing 1 or 10 reactions with 100 µg of antibodies or other proteins with a molecular weight of Mr ~150 kDa and microcolumns for purification of the target molecule from unreacted reagents by gel filtration.

*Important!* To label proteins with substantially different molecular weights, to obtain a labeling degree of 3–5, it is necessary to take 100 µg × Mr (protein) / 150 kDa per reaction.

# Kit components

| Kit component | Count | |
|---|---|---|
| | BB21-1rxn 1 reactions | BB21-10rxn 10 reactions |
| S6115, Desalting receptacle vial, 1.5 mL, 1 pcs | 1 | — |
| A8115, Desalting spin column with waste vial, 1 pcs | 1 | — |
| 16115, Desalting receptacle vial, 1.5 mL, 10 pcs | — | 1 |
| 18115, Desalting spin column with waste vial, 10 pcs | — | 1 |
| 2897-10nmol, Biotin-XX-NHS ester, 10 nmol | 1 | 10 |
| 11125, PBS tablet, for 100 mL of buffer, 1 pcs | 1 | 1 |
| 15050, DMSO (dimethyl sulfoxide), labeling grade, 1 mL | 1 | 1 |
| 1584-05mL, Sodium azide solution, 3%, 0.5 mL | 1 | 1 |
| 1689-15mL, Sodium bicarbonate, 126 mg | 1 | 1 |

Store at temperature between 4 °C and 20 °C. Do not freeze! Transportation: at room temperature for up to 3 weeks. Avoid prolonged exposure to light. Desiccate.

Shelf life 12 months.

# Protocol

## 1. Protein Preparation

- The target protein (antibodies, etc.) must not contain free amino acids, other proteins (e. g., BSA), or buffer components with pH 2–7.5 or 9–12.
  If the protein is in a sodium bicarbonate buffer with pH 8–8.5 and is guaranteed to be free of other impurities, it can be used directly in the reaction without additional purification.
- If you are not certain that the antibody preparation is free of impurities, purification is mandatory. For protein purification, one of the following methods is recommended: dialysis, gel filtration, or ultrafiltration using 0.1 M sodium bicarbonate solution*.
- Optimal labeling conditions: protein concentration 1 mg/mL in 0.1 M sodium bicarbonate solution without extraneous impurities. The presence of sodium azide as a preservative (up to 0.04%) does not affect the reaction.
- If the protein concentration is below 1 mg/mL, concentrate it to 1 mg/mL by ultrafiltration followed by dilution. For complete removal of potential impurities, two concentration/dilution cycles are recommended. After each concentration step, dilute the protein with 0.1 M sodium bicarbonate.
- If the protein concentration is above 1 mg/mL, it is recommended to perform a single wash on an ultrafiltration column using 0.1 M sodium bicarbonate before dilution. For complete removal of possible impurities, two concentration/dilution cycles are recommended. After washing, dilute the protein with 0.1 M sodium bicarbonate.
- Protein concentration should be monitored spectrophotometrically (a cuvette-free spectrophotometer is recommended).

*Important!* Protein concentration in the reaction mixture affects the degree of labeling. For example, when reacting 100 µg of protein in a volume less than 100 µL, the modified protein will have a degree of labeling greater than 5 biotin molecules per protein molecule. When reacting 100 µg of protein in a volume greater than

100 µL, the modified protein will have a labeling degree of fewer than 3 biotin molecules per protein molecule.

* To prepare 0.1 M sodium bicarbonate, add 15 mL of deionized water to the vial containing dry sodium bicarbonate supplied in the kit.

## 2. Protein Labeling

Add the protein solution in 0.1 M sodium bicarbonate (recommended amount: 100 µg** of protein in 100 µL) to the tube containing the lyophilized biotinylation reagent. Vortex until the reagent is completely dissolved and incubate for 1 hour at room temperature or overnight at 4 °C.

** If it is necessary to perform the reaction with a smaller amount of protein, dissolve the lyophilized reagent in 10 µL of anhydrous DMSO and use 1 µL of this solution per 10 µg of protein. The reagent solution in DMSO is not suitable for storage.

## 3. Removal of Excess Reagent

1. Dissolve the PBS buffer tablet in 100 mL of water.
2. Prepare the column. Ensure the column is at room temperature. Resuspend the resin by vortexing. Remove the caps, place the column into a collection tube, and centrifuge for 2 minutes at 1000 × *g* (strictly observe the specified speed; for a standard rotor with a 6 cm radius, 1000 × *g* corresponds to 3800 rpm). During centrifugation, maintain proper orientation of the column in the rotor: the protrusion at the top of the column must face away from the rotor center. Discard the filtrate after centrifugation.
3. Apply 400 µL of PBS to the column and centrifuge for 2 minutes at 1000 × *g*. Discard the filtrate.
4. Transfer the column to the 1.5 mL tube for collection of the biotinylated protein supplied in the kit.
5. Apply 100 µL*** of the reaction mixture to the center of the column, incubate for 1 minute, and centrifuge for 2 minutes at 1000 × *g*. The purified biotinylated protein**** will be collected in the tube.

*** For purification, 50–100 µL can be applied to the column. If the reaction was performed in a smaller volume, adjust the volume to at least 50 µL with PBS buffer before purification.

**** After centrifugation, sodium azide solution (provided in the kit) may be added to the protein at a final concentration of 1% of the sample volume. If necessary, the protein preparation may be aliquoted during centrifugation. In this case, the working aliquot should be stored at 4 °C, while the remaining aliquots should be stored at -20 °C. Only solutions containing sodium azide may be stored at 4 °C.

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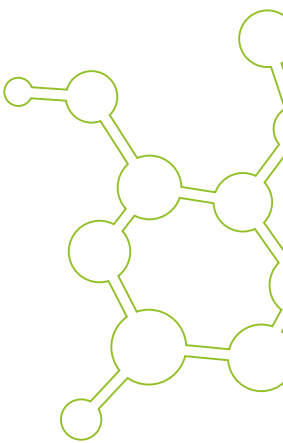

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