

# MEBEP TECH(HK) Co., Limited

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## **NHS-Biotin**

### Product Number: BC-NB-25mg

### Description

D-Biotin N-hydroxysuccinimide ester (NHS-Biotin) is the most popular amine reactive biotin derivative used to modify proteins and other biomolecules under mild conditions. NHS-Biotin has cell membrane permeability and can be used for covalent labeling of intracellular proteins, as well as commonly used for protein detection. When used in combination with streptavidin and immobilized streptavidin, it is used for various biotin streptavidin binding experiments.

### Protocol

- 1. Before opening the bottle, remove the product from the refrigerator and let it equilibrate at room temperature for at least 20 minutes.
- 2. Dissolve the protein in 100mM sodium phosphate, 150mM NaCl, pH 7.2-7.5 or other suitable amine free buffer to a concentration of 1-10mg/mL.

Note: If the protein contains preservatives such as sodium azide or small molecule stabilizers, the protein needs to be dialyzed into an amine free buffer to remove impurities; If the protein is soluble in Tris or glycine buffer, it needs to be dialyzed into amine free buffer to remove impurities.

- 3. Once used, prepare 20mg/mL NHS-Biotin with anhydrous DMF or DMSO
- 4. Add sufficient NHS-Biotin storage solution to the protein solution, so that the excess molar ratio of NHS-Biotin to protein reaches 10-20 times, and mix thoroughly.

Note: To ensure optimal results, the first experiment can be tested at a ratio of 5:1, 10:1, 15:1, 20:1 excess molar ratio to determine the optimal ratio; Ensure that the content of DMSO or DMF in the final labeling system is less than 10% of the total volume.

- 5. Incubate at room temperature for 30-60 minutes, or at 4°C for>2 hours, while the reaction solution is continuously shaken.
- 6. The biotinylated labeled protein was purified by desalting through dialysis or resin gel column.

Note: The biotinylated marker protein needs to be stored in a buffer containing carrier protein (such as 0.1% BSA) at a concentration>0.5mg/mL; For long-term preservation, biotinylated labeled proteins can be freeze-dried or packaged into single doses and stored at -80°C.

### Note

- NHS-Biotin is reconstituted in DMSO or DMF to prepare a storage solution (e.g. 10mg/mL). If it cannot be used up in a single dose, it should be stored at< -20°C and used up within 2 weeks;</li>
- 2. For your safety and health, please wear lab coats and disposable gloves when operating.