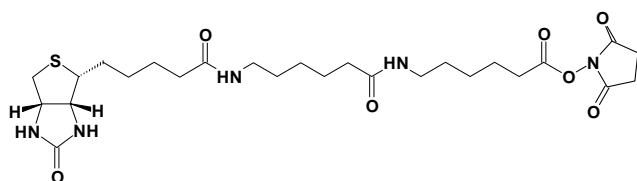


Biotin-XX Protein Labeling Kit Protein Biotinylation Kit

Cat. No.	Amount
FP-322	5 reactions

For *in vitro* use only!



molecular structure of biotin-XX NHS-ester

Kit Contents

- **Biotin-XX NHS-ester**
5 vials containing 1 mg each
- **Dimethylformamide (DMF)**
500 µl
- **Sodium bicarbonate**
1 vial containing 168 mg
- **ultra-pure water**
2 ml

Storage and Stability

Upon receipt, store the biotin-XX at -20°C. The other components may be stored at room temperature. If stored as recommended, Jena Bioscience guarantees optimal performance of this product for 12 months.

Introduction

Binding of biotin by avidin, streptavidin or NeutrAvidin™ is the strongest known biological interaction with a dissociation constant in the range of 10⁻¹⁵ M.

The vitamin biotin may be conjugated to many proteins without loss of their biological activity due to the small size of the biotin molecule. The biotinylated probe is usually detected by avidin, streptavidin or NeutrAvidin™, carrying a reporter group, e.g. horseradish peroxidase (HRP) or a fluorescent label. Fluorescent streptavidin (Cat.# FP-303) and avidin (Cat.# FP-304) assay kits are available from Jena Bioscience.

The protein of interest (POI) is often biotinylated at different positions. Each conjugated biotin binds one molecule of avidin, streptavidin or NeutrAvidin™, resulting in signal amplification.

Biotin-XX inserts a C12-spacer between the biotin and the protein molecule, hence reduces steric hindrance and increases accessibility for avidin, streptavidin or NeutrAvidin™.

The interaction between biotin and biotin-binding proteins is used in various applications such as affinity chromatography, fluorescence-activated cell sorting (FACS), ELISA and Western Blot.

This Protein Biotinylation Kit contains all reagents required for performing 5 separate labeling reactions of 5 mg of POI.

Protocol

General notes

The protein concentration should be at least 2 mg/ml, higher concentrations – up to 10 mg/ml – are preferable since labeling efficiency suffers from low concentrations. We recommend using about 5 mg protein per labeling reaction.

Buffers containing primary amines such as Tris and glycine are not suitable for the labeling reaction and must be exchanged with suitable amine-free buffer such as PBS, MES, or HEPES before starting the labeling reaction.

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Experimental protocol

1. Dissolve the sodium bicarbonate by adding 2 ml ultra-pure water. The resulting 1 M solution is stable at 4°C for at least 2 weeks.
2. Add the appropriate volume of sodium bicarbonate (1 M) to your protein solution to achieve a final concentration of 100 mM.
3. Remove one biotin-XX vial from the freezer and equilibrate it to room temperature before opening. Prepare the biotin-XX by adding 100 µl ultra-pure water resulting in a concentration of 10 mg/ml. Vortex until the biotin-XX is completely dissolved! Prepare the solution shortly prior to use!
4. Add 500 µl protein solution (10 mg/ml) and the dissolved biotin-XX to an appropriate vial. Vortex carefully and centrifuge briefly to collect the reaction mixture at the bottom of the tube.
5. Incubate for one hour in a shaker at room temperature.
6. Purify the conjugate using standard gel filtration columns such as Sephadex G-25 or similar. Alternatively, the free biotin-XX may be separated from the conjugate by dialysis or appropriate spin concentrators.

Please note that protein purification materials are not provided with the kit!

7. Analyze your conjugate by SDS-PAGE and/or Dot blot using the fluorescent streptavidin (Cat.# FP-303) or avidin (Cat.# FP-304) assay kit.

Storage of the Conjugate

Store the conjugate just like the unlabeled protein. We recommend dividing the solution into small aliquots and freeze at -20°C or -80°C. Avoid repeated freezing and thawing!

Troubleshooting

Low biotinylation efficiency

- Concentration of protein solution
The assay is optimized for labeling of 5 mg protein at a concentration of 10 mg/ml. The efficiency of biotinylation is strongly concentration dependent and varies among different proteins. Thus, in every single case optimization might be necessary to obtain optimal biotinylation.
- Buffer composition
Protein solutions containing primary amines (even traces thereof) dramatically decrease biotinylation efficiency. Make sure that your protein is extensively dialyzed in case it has been in contact with amine-containing substances.
- Impact of the pH
Check the pH of your protein solution! The reference range is 8.2 – 8.5.
The primary amino groups of the protein must not be protonated to be reactive thus, the pH of the protein solution has to be sufficiently high. On the other hand, the hydrolysis rate of NHS esters increases with the pH of the solution, resulting in non-reactive biotin-XX. Optimal biotinylation results have been obtained at pH 8.3.
100 mM sodium bicarbonate might not be sufficient to raise the pH to optimum in strongly buffered protein solutions at a lower pH. You may add more sodium bicarbonate until the optimal pH is achieved.

Note that biotinylation efficiency not only depends on the surrounding conditions but also on the protein characteristics. The tertiary structure and the resulting number of lysines on the surface of the protein play a role as well as the isoelectrical point and the behaviour of the protein at pH 8.3.

For further information and related products, please visit us at: www.jenabioscience.com