

# Biotinylation of Antibodies

*For biotinylation with Biotin-NHS*

This is a protocol for biotinylation of antibodies based on standard methods. For technical specifications and further details on the biotinylation, please follow the instructions provided by the chosen manufacturer's biotinylation reagents.

## Introduction

The most common biotinylation reagent is an N-hydroxysuccimide (NHS)-activated biotin, which reacts with primary amine groups from the side chain of Lysine residues and the N-terminus of each polypeptide chain in the antibody.

## Protocol

This is an example protocol including the biotinylation reaction and purification of the resulting biotinylated antibodies.

### 1. Preparation of the biotin-NHS stock

1.1. Prepare a stock solution of Biotin-NHS with a concentration of 40 mg/mL in dry DMSO.

### 2. Biotinylation

2.1. Add stock biotin-NHS to antibody solution, according to the table:

Antibody concentration	Quantity of stock Biotin-NHS
1 mg/mL or higher	2 $\mu$ L of stock per <b>mg antibody</b>
Lower than 1 mg/mL	2 $\mu$ L of stock per <b>mL antibody solution</b>

2.2. Stir the reaction mix at room temperature in the dark, for 2 hours.

2.3. Add the same volume as the biotin-NHS stock of 0.5 mol/L of ethanolamine to the reaction mix and incubate for 30 minutes. This will block the remaining active biotin-NHS.

### 2.4 EXAMPLE

For an antibody concentration of: 0.5 mg/mL in a volume of 3 mL

2.4.1. Prepare the biotin-NHS stock in DMSO, as indicated in step 1

2.4.2. Add the stock biotin-NHS in DMSO. In this case, the concentration is less than 1 mg/mL, so add 6  $\mu$ L stock biotin-NHS (2  $\mu$ L stock/mL antibody solution)

2.4.3. Stir the reaction mix at room temperature for 2 hours in the dark

2.4.4. Add 6  $\mu$ L of 0.5 mol/L ethanolamine to the reaction mix

2.4.5. Incubate for 30 minutes

For an antibody concentration of: 1.5 mg/mL in a volume of 3 mL

2.4.6. Prepare the biotin-NHS stock in DMSO, as indicated in step 1

2.4.7. Add the stock biotin-NHS in DMSO. In this case, the concentration is more than 1 mg/mL, so add 9  $\mu$ L stock biotin-NHS (2  $\mu$ L stock/mg antibody)

2.4.8. Stir the reaction mix at room temperature for 2 hours in the dark

2.4.9. Add 3  $\mu$ L of 0.5 mol/L ethanolamine to the reaction mix

2.4.10. Incubate for 30 minutes

### 3. Purification

#### For smaller volumes, (<2.5 mL)

1. Filter the reaction mix with small disposable columns (e.g. PD10, NAP 10, NAP 5 from GE Healthcare Life Sciences).
2. Repeat to remove as much free biotin as possible

#### For larger volumes (>2.5 mL)

Filter the reaction mix by gel filtration / buffer exchange with G25 media (e.g. Sephadex G-25 fine, GE Healthcare Life Sciences). This is the quickest and easiest method to remove the free biotin.

## Important notes on biotinylation of antibodies

- In some antibodies, it is possible that the Lysine residues are essential for antigen binding. In these cases, binding with the antigen is reduced or lost with biotinylation. To overcome this issue, it is possible to reduce the amount of biotin-NHS used. Alternatively, other chemistries such as carbohydrate coupling with a biotin hydrazide may be used.
- NHS-activated biotins react efficiently with primary amino groups in pH 7-9 buffers to form stable amide bonds. In general, PBS pH 7.4 or 100 mol/L carbonate buffer with pH 8.0 or pH 8.4 are used for the biotinylation of antibodies, but the pH can be optimized further if required.

Because NHS reacts with amine groups, these must not be present in the buffer. Therefore, the antibody must not be in Tris buffer or contain sodium azide ( $\text{NaN}_3$ ), since they will block conjugation.