

# Introduction

Sulfo-NHS-LC-Biotin is an N-hydroxysuccinimide ester of biotin that readily couples to primary amine groups of proteins. Biotinylated proteins can be used as capture reagents in MSD immunoassays using streptavidin- or avidin-coated plates. The conjugates are stable, may be used at low concentrations, and have low non-specific binding. Large batches of stable conjugates can be prepared, validated, and used for long periods of time. The conjugation procedure is simple, making the product ideal for molecules that contain primary amines (e.g., lysine in proteins).

This guide describes the biotin conjugation protocol for proteins with a molecular weight (MW) > 40,000 Da. The straightforward procedure involves an optional buffer exchange prior to conjugation, a 2-hour incubation step, and a buffer exchange post-conjugation to quickly isolate the conjugated protein using a spin column. Smaller proteins/polypeptides may also be conjugated using Sulfo-NHS-LC-Biotin if they have an accessible lysine or N-terminal amino group; however, alternative conjugation parameters and separation methods may be needed to remove unconjugated biotin.

## Biotin Conjugation Pack Components

Reagent	Storage	Size	Quantity	Description
EZ-Link Sulfo-NHS-LC-Biotin, No-Weigh Format	≤-20°C	1 mg	8 vials	Sulfo-NHS Biotin label for coupling to antibodies and other proteins
Zeba Spin Desalting Column, 40K MWCO	2-8°C	0.5 mL	10 columns	Size exclusion chromatography columns for the purification of proteins larger than 40,000 Da
Filter, 0.22 µm	RT	N/A	10 each	Filter for post-conjugation purification
Syringe, 1 mL	RT	N/A	10 each	Syringe for post-conjugation purification
Conjugation Buffer	RT	40 mL	1 bottle	100 mM Phosphate Buffer, pH 7.9
Conjugate Storage Buffer	RT	40 mL	1 bottle	Phosphate-buffered saline (PBS), pH 7.4, with 0.05% sodium azide

## Additional Materials (not provided)

1. Polypropylene microfuge tubes and 15 mL conical tubes
2. Protein assay such as BCA, Bradford, or Lowry
3. Concentrator (optional) (e.g., MilliporeSigma BIOMAX-50, AMICON Ultra-4, or AMICON Ultra-15 concentrators)
4. Biotin Quantification Kit (e.g., Pierce Biotin Quantification Kit or FluoReporter Biotin Quantitation Assay Kit from Thermo Fisher Scientific)
5. Additional Zeba Spin Desalting Columns, 40K MWCO are available in various sizes from Thermo Fisher Scientific (catalog numbers 87766 – 87773)

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## Quick Guide

# Conjugation Protocol

### Pre-Conjugation Procedure

1. Prepare a 1-2 mg/mL solution of the protein to be conjugated in the supplied Conjugation Buffer.  
Notes:
  - Preservatives such as sodium azide or EDTA, buffer components containing primary amines (e.g., Tris, glycine), and glycerol, must be removed by buffer-exchange using the supplied Zeba Spin Desalting Columns before starting the conjugation reaction. Note: Conjugate Storage Buffer should not be used at this stage.
  - Filter the protein using a 0.2  $\mu\text{m}$  filter.
  - Measure the concentration of the protein solution to be conjugated. Protein concentration can be calculated from an  $\text{OD}_{280}$  absorbance or with a protein concentration assay.
  - Proteins should be in a carrier-free formulation for conjugation; carrier proteins such as BSA and gelatin cannot be removed by buffer exchange.
2. Equilibrate the protein to be conjugated at the conjugation temperature of 23°C (20-25°C is acceptable).
3. Calculate the amount of Sulfo-NHS-LC-Biotin stock solution required using the formula below.

### Calculations

$$1,000 \times \frac{\text{Protein conc. (mg/mL)} \times \text{Challenge ratio} \times \text{Vol. of protein solution } (\mu\text{L})}{\text{Protein MW (Da)}} = \text{nmol of Sulfo-NHS-LC-Biotin reqd.}$$

Using this value, calculate the volume of Sulfo-NHS-LC-Biotin stock solution required for the reaction. The default Sulfo-NHS-LC-Biotin stock concentration to use is 0.5 nmol/ $\mu\text{L}$ .

$$\frac{\text{nmol of Sulfo-NHS-LC-Biotin reagent required}}{\text{Conc. of Sulfo-NHS-LC-Biotin stock solution (nmol}/\mu\text{L})} = \mu\text{L of Sulfo-NHS-LC-Biotin stock soln. reqd}$$

### Conjugation Procedure

1. Gently tap the Sulfo-NHS-LC-Biotin vial. One lyophilized vial has 1 mg of Sulfo-NHS-LC-Biotin (MW 556.6 g/mol), which equals 1,800 nmol. Immediately prior to use, reconstitute the vial of Sulfo-NHS-LC-Biotin with 180  $\mu\text{L}$  of cold distilled water to generate a stock solution of 10 nmol/ $\mu\text{L}$ . Mix gently.
2. Add 10  $\mu\text{L}$  of the above 10 nmol/ $\mu\text{L}$  stock solution to 190  $\mu\text{L}$  of cold distilled water to generate a working stock solution of 0.5 nmol/ $\mu\text{L}$ . Gently vortex.
3. Add the calculated volume (Pre-Conjugation Procedure, Step 3) of reconstituted Sulfo-NHS-LC-Biotin to the protein solution and vortex immediately. Discard any remaining Sulfo-NHS-LC-Biotin. Note: For most applications using IgG antibodies, optimal performance is obtained with challenge ratios between 5:1 and 20:1. Incubate at 23°C for 2 hours (20-25°C is acceptable). Consistent conjugation conditions should be maintained between multiple preparation lots to ensure reproducibility.

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## Conjugation Protocol

### Post-Conjugation Procedure

1. Prepare Zeba Spin Desalting Columns, 40K MWCO. Remove the column's bottom closure and loosen the cap. **Do not remove the cap.** Place the column in a collection tube to remove the storage buffer and wash the column 3 times with MSD Conjugate Storage Buffer. Each preparation step should be carried out by centrifuging the columns (and their respective collection tubes) in a centrifuge with a swinging bucket rotor at 2-8°C (for 2 mL Zeba columns) or a tabletop microcentrifuge (for 0.5 mL Zeba columns).

Table 1: Specifications for 0.5 mL and 2 mL Zeba Spin Desalting Columns, 40K MWCO

Size of Column	0.5 mL	2 mL	
Sample Volume Range	70-100 $\mu$ L	200-450 $\mu$ L	
Wash Buffer Volume	300 $\mu$ L	1,000 $\mu$ L	
Sample Volumes to use as stacker*	N/A	< 350 $\mu$ L	
Optional Stacker Volume	N/A	40 $\mu$ L	
Centrifugation Speed	1,500 x g	1,000 x g	
Centrifugation Time (Min)	Storage Solution Removal	1	2
	Wash 1	1	4 - 8
	Wash 2	1	2 - 8
	Wash 3	3	5 - 8
	Sample Recovery	3 - 4	6 - 8

\* When using the indicated sample volumes, use a stacker volume to achieve highest recovery. Stacker volume should be added after addition of the protein to the column.

2. Apply the conjugation reaction to the center of the spin column in a drop-wise manner (refer to Table 1 for sample volume). Centrifuge the columns in clean, new collection tubes to separate the biotin conjugated protein from unconjugated biotin. The biotin conjugated protein will be present in the eluate. Retain the purified conjugated material in the collection tubes and discard the columns.
3. Filter the conjugated protein using a 0.2  $\mu$ m filter.
4. Calculate final conjugated protein concentration in mg/mL by measuring  $A_{280}$  and dividing by the extinction coefficient of the protein at 280 nm. If the path length of the spectrophotometer is not 1 cm, multiply the extinction coefficient by the path length (in cm). Alternatively, a protein quantitation assay such as BCA, Bradford, or Lowry assay can be used to calculate protein concentration.
5. Determine the Biotin label:protein conjugation ratio by measuring the molar biotin concentration of the conjugate using a Biotin Quantification Kit then divide by the molar protein concentration of the conjugate.
6. Antibody conjugates are usually stable for at least 1 year at 2-8°C in conjugate storage buffer. Stability of other proteins needs to be determined by the user.

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# Quick Guide

## Worksheet

### Pre-Conjugation Calculations

$1,000 \times \frac{\text{Protein conc. (mg/mL)} \times \text{Challenge ratio} \times \text{Vol. of protein solution } (\mu\text{L})}{\text{Protein MW (Da)}} = \text{nmol of Sulfo-NHS-LC-Biotin reagent reqd.}$

$\frac{\text{nmol of Sulfo-NHS-LC-Biotin reagent required}}{\text{Conc. of Sulfo-NHS-LC-Biotin stock solution (nmol}/\mu\text{L)}} = \mu\text{L of Sulfo-NHS-LC-Biotin stock solution required}$

### Conjugation Procedure

Sample concentration: \_\_\_\_\_ Buffer exchange: Y / N

Volume of Sulfo-NHS-LC-Biotin stock solution added to protein: \_\_\_\_\_

Time reaction started: \_\_\_\_\_ Time reaction completed: \_\_\_\_\_

Separation of conjugated material: \_\_\_\_\_

Column size: \_\_\_\_\_ Buffer: \_\_\_\_\_

### Post-Conjugation Calculations

Protein assay: \_\_\_\_\_ Protein conc. (mg/mL): \_\_\_\_\_ OD<sub>280</sub>: \_\_\_\_\_

$\frac{\text{Protein conc. (mg/mL)}}{\text{Protein MW (Da)}} = \text{M (A)}$

Molar concentration of biotin determined in biotin quantitation assay = \_\_\_\_\_ M (B)

Labeling incorporation ratio (Sulfo-NHS-LC-Biotin label:Protein) = (B / A) \_\_\_\_\_

### Contact Information

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