MagicLink™ Biotin Antibody Labeling Kit: Sulfo-NHS-Biotin

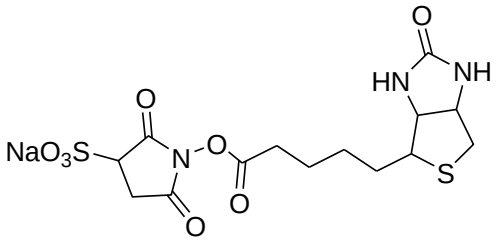
Components

Component		Product size		Storage
		BP-50056	BP-50057	
		8 rxn kit	8 rxn	
A	Sulfo-NHS-biotin	8 x 2 mg	8 x 2 mg	-20C
B	Reaction Buffer	30 ml	N/A	RT
C	Protein concentrator	8	N/A	RT
Note: BP-50057, user may use 1x PBS pH 7.2 – 7.4 as reaction buffer is not supplied.				

Overview

MagicLink™ Sulfo-NHS-Biotin Antibody Biotinylation Kit provides optimized reagents for labeling antibodies and desalting columns for purifying the labeled molecule. Each reaction is sufficient for labeling 50-200 µg of antibody in 100 µl reaction volumes. The link arm length has 13.5A and the water-soluble group ($NaSO_3$-NHS) will leave the biotinylated molecules.

This kit is specifically optimized to label antibodies at a scale for 1 mg. The kit format is a convenient single-use microtubes, eliminating difficulties associated with weighing small quantities of reagent. Biotin is a small, naturally occurring vitamin that binds with high affinity to avidin and avidin-like proteins. Biotinylated antibodies typically retain biological activity because the biotin group is relatively small. An antibody conjugated with several biotin molecules can interact rapidly and tightly with streptavidin. Sulfo-N-Hydroxysuccinimide (NHS) esters are the most popular biotinylation reagents. In pH 7-9 buffers, NHS esters react efficiently with primary amino groups (-NH2) by nucleophilic attack, forming an amide bond and releasing the NHS (Figure 1). Proteins typically have many sites for labeling, including the primary amine in the side chain of lysine (K) residues and the N-terminus of each polypeptide.



Sulfo-NHS-biotin
Mol. Wt.: 443.43
Spacer arm: 13.5A

At a Glance

Protocol summary

1. Add 200 µl H_2O to Sulfo-NHS-Biotin vial.
2. Prepare the antibody at 2 mg/ml by pH 7.4 PBS buffer.
3. Add amount calculated Biotin working solution to antibody stock solution.
4. Incubate at room temperature for 30 - 60 minutes.
5. Remove Excess Biotin reagent by spin column.

Note: Upon receipt, store the kit at 4°C. When stored properly, the kit should be stable for six months. Alternatively, components Sulfo-NHS-Biotin can be stored at-20°C. Do not freeze reaction buffer (component B). Warm all the components and centrifuge the vials briefly before opening, and immediately prepare the required solutions before starting your conjugation. The following protocol is an example for labeling goat anti-mouse IgG antibody.

Preparation of Working Solution

1. Calculation

   The degree of labeling (DOL) depends on the size and distribution of amino groups on the protein and the amount of biotin reagent used. Compared to reactions involving concentrated protein solutions, labeling reactions with dilute protein solutions require a greater fold molar excess of biotin reagent to achieve the same incorporation level. Experiments that used a 20-fold molar excess of biotin reagent to label 1-5 mg/ml antibody (IgG) resulted in 4-6 biotin groups per antibody molecule. Experiments that used a 50-fold molar excess of biotin reagent to label 50-200 µg of antibody (in 200-700 µl) resulted in 1-3 biotin groups per antibody molecule. Adjust the molar ratio of Sulfo-NHS-Biotin to protein to obtain the desired level of incorporation.

   a. Sulfo-NHS-Biotin working solution: Add 200 µl Di-H_2O into the one vial of Sulfo-NHS-Biotin.
   b. Calculate volume of Sulfo-NHS-Biotin stock solution to add to the reaction for a 20-fold molar excess

      Formula:

Vbiotin (ul) = Biotin amount (mmol) * MW Biotin NHS (mg/mmol) * (V biotin (µl) /2 mg) = Biotin amount (mmol) * 443 mg/mmol * 200 µl/2 mg = 20 * protein amount (mmol) * 443 mg/mmol*200 µl/2 mg = 20* Vprotein (ml) * Conc protein * (mg/ml) / MW protein * 443 mg/mmol * 200 µl/2 mg

      Example:

      For 1 ml of a 2 mg/ml IgG (150,000 MW) solution, 11.8 µl of Sulfo-NHS-Biotin will be added.

      V_{biotin}(ul) = 20 * Vprotein (ml) * Conc protein * (mg/ml) / MW protein * 443 mg/mmol * 200 µl/2 mg
      = 20 * 1 * 2 / 150000 * 443 * 200 / 2
      = 11.8 µl

2. Antibody working solution:

   For labeling 1 mg antibody, the preferred concentration antibody concentration is 2 mg/ml

   Note:

   - If you have a different concentration, adjust the antibody concentration accordingly.
   - The antibody should be dissolved in 1X phosphate buffered saline (PBS), pH7.2-7.4; If the antibody is dissolved in glycine buffer, it must be dialyzed against 1X PBS, pH 7.2-7.4, or use Amicon Ultra-0.5, Ultracel-10 Membrane, 10K MWCO (Cat # UFC501008 from Millipore) to remove free amines or ammonium salts (such as ammonium sulfate and ammonium acetate) that are widely used for antibody precipitation.
   - Impure antibodies or antibodies stabilized with bovine serum albumin (BSA) or gelatin will not be labeled well.
   - The conjugation efficiency is significantly reduced if the antibody concentration is less than 1 mg/ml. For optimal labeling efficiency the final antibody concentration range of 1-5 mg/ml is recommended.

Sample Experimental Protocol

1. Add biotin amount needed (as calculated above) to the antibody solution antibody.
2. Mix by gently pipetting up and down.
3. Incubate the reaction at room temperature for 30 minutes.

Excess Biotin Removal

1. Hydrate concentrator membrane 'filter device' with 400 to 500 µl of reaction buffer or DI water, and microcentrifuge 14,000 x g, for 3 minutes. Discard, liquid from filter device and collection tube.
2. Spin down by adding biotin labeled antibody to the concentrator/filter device up to 500 µl. Microcentrifuge at 14,000 x g, 8 minutes, or to minimum volume ~ 50 µl left in the filter device. Discard waste from the collection tube.
3. Desalt by adding reaction buffer to the filter device up to 500 µl. Microcentrifuge at 14,000 x g, 8 minutes, or to minimum volume ~ 50 µl left in the filter device. Discard waste from the collection tube.
4. Repeat step 3, twice.
5. Collect labeled antibody from filter device into a microcentrifuge tube.
6. Optional for maximum recovery, add reaction buffer, volume determined by the user, to the filter device to rinse out residual antibody, microcentrifuge pulse spin, collect antibody/reaction buffer from filter device, add to the microcentrifuge tube from step 5, mix.
7. Store biotinylated antibody at 4°C for < 1 month. For longer periods, store at -20°C or -80°C (e.g., 0.1% bovine serum albumin) and 0.02-0.05% sodium azide.

Biotin Quantitation

Use Pierce's Biotin quantitation kit to calculate the degree of labeling (DOL). See Pierce 285005 for details.

Storage of Antibody-Biotin

The antibody conjugate should be stored in the presence of a carrier protein.

Troubleshooting

Problem	Possible cause	solution
Low or no biotinylation	Buffer containing primary amine	Buffer exchange the antibody into a non-amine-containing buffer such as the PBS provided by desalting columns or dialysis
	NHS-PEG4-biotine was hydrolyzed	Use reagent immediately upon reconstitution
	Carrier protein was present in the antibody solution	Remove carrier protein before biotinylation by using Protein A, G or A/G resin or an antibody clean-up kit. This will reduce competition for labeling