MAGICLINK ™ NHS PEG4 Antibody Biotinylation Kit

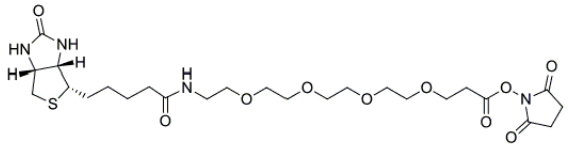
Components

Component		Product size		Storage
		BP-50054	BP-50055	
		8 rxn kit	8 rxn	
A	NHS-PEG4-Biotin	8 x 2 mg	8 x 2 mg	-20 °C
B	Reaction Buffer	30 ml	N/A	4-8 °C
C	Protein concentrator	8	N/A	RT

Note:

- BP-50054 and BP-50055 is shipped at ambient temperature. Upon receipt, store components A NHS-PEG4-Biotin at- 20°C, and component B reaction buffer at 4-8oC.
- BP-50055, user may use 1x PBS pH 7.2 – 7.4 as reaction buffer is not supplied.
- Warm all the components and centrifuge the vials briefly before opening, and immediately prepare the required solutions before starting your conjugation. The following SOP is an example for labeling goat anti-mouse IgG antibody.

Overview



NHS-PEG$_4$-Biotin
MW: 588.67
Spacer Arm: 29.0A

MAGICLINK ™ NHS PEG4 Antibody Biotinylation Kit provides optimized reagents for labeling antibodies and protein concentrator for purifying the labeled molecule. Each reaction is sufficient for labeling 50-200 µg of antibody in 100 µl reaction volumes. The featured hydrophilic polyethylene glycol (PEG) increases the reagent solubility. Consequently, antibodies labeled with NHS-PEG4-Biotin exhibit less aggregation when stored in solution compared to antibodies labeled with reagents having only hydrocarbon spacers.

This kit is specifically optimized to label antibodies at a scale up to 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. Antibodies conjugated with several biotin molecules can interact rapidly and tightly with streptavidin.

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. 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.

At a Glance

Protocol summary

1. Add 200 µl DI H_2O to NHS-PEG4-Biotin vial
2. Prepare the antibody at 2 mg/ml in reaction buffer or PBS pH7.4.
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 concentrators.

Preparation of Working Solution

1. Calculation

The degree of labeling 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 NHS-PEG4-Biotin to protein to obtain the desired level of incorporation.

a. NHS-PEG4-Biotin working solution: Add 200 µl DI-H_2O into the one vial of NHS-PEG4-Biotin.

b. Calculate volume of NHS-PEG4-Biotin to add to the reaction for a 20-fold molar excess.

Formula

$$V\_biotin\_NHS(ul) = \frac{Amount\_biotin(mmol) \times MW\_biotin\_NHS(mg/mmol)}{Conc\_biotin\_NHS\ (mg/ul)}$$

$$= 20\ x\ \frac{Amount\_protein(mmol) \times MW\_biotin\_NHS(mg/mmol)}{Conc\_biotin\_NHS\ (mg/ul)}$$

$$= 20\ X\ \frac{Vprotein(ml) \times Conc\_protein\ (mg/ml)}{MW\_protein\ (mg/mmol)} x\ \frac{MW\_biotin\_NHS\ (mg/mmol)}{Conc\_biotin\_NHS\ (mg/ul)}$$

$$= 20\ X\ \frac{Vprotein(ml) \times Conc\_protein\ (mg/ml)}{MW\_protein\ (mg/mmol)} x\ \frac{589(mg/mmol)}{2/200\ (mg/ul)}$$

Example:

For 1 ml of a 2 mg/ml IgG (MW 150,000) solution, 15.7 µl of NHS-PEG4 biotin will be added.

$$V\_biotin\_NHS(ul) = 20 * 1 * 2 / 150000 * 589/(2 / 200)$$

$$= 15.7\ \mu l$$

2. Prepare 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 reaction buffer or 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.

Conjugation Experimental Protocol

Conjugation step

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

De-salt step

Note: the step is to remove excess biotinylation reagent and other side products.

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 a small amount of 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, optional with stabilizing protein (e.g., 0.1% bovine serum albumin) and 0.02-0.05% sodium azide.

Biotin quantitation

Use BroadPharm's Biotin quantitation kit to calculate the degree of labeling (DOL), Cat# BP-50060.

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 reaction buffer provided, using protein concentrator or 1x PBS by 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