MAGICLINK™ Site Specific Biotinylation Kit

Components

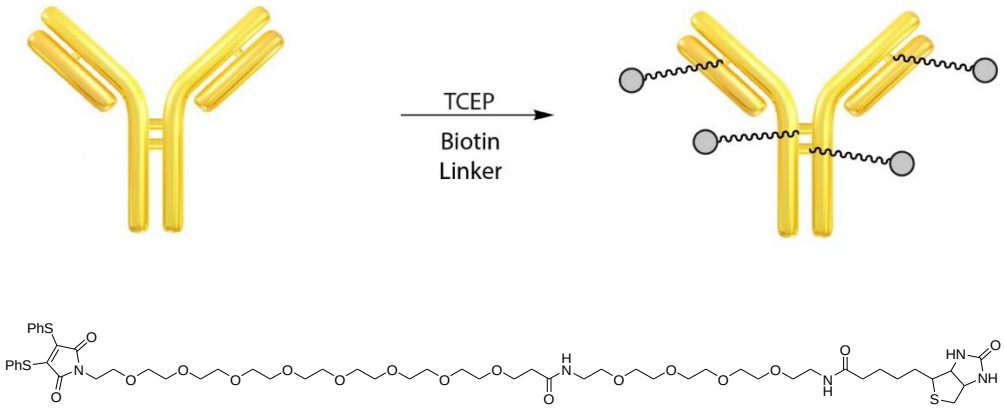
Component		Product size				Storage
		BP-50123	BP-50124	BP-50125	BP-50126	
		3 x 100 µg kit	8 x 100 µg kit	1 x 1 mg kit	3 x 1 mg kit	
A	Biotin Linker (MW ~ 1200)	3 x 100 µg	8 x 100 µg	1 x 1 mg	3 x 1 mg	-20 °C
B	Reaction Buffer pH8	30 ml	30 ml	30 ml	30 ml	4-8 °C
C	Protein concentrator	3	8	1	3	Room temp.
D	DMSO	1	1	1	1	Room temp.
E	TCEP.HCl	3 x 16 µg	8 x 16 µg	1 x 160 µg	3 x 160 µg	-20 °C

Note:

- Product is shipped at ambient temperature. Upon receipt, store components as indicated in the table above.

Overview

MAGICLINK™ Site Specific Biotinylation Kits provide optimized reagents for biotinylating antibodies.
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. Current bintinylation methods, such as amine-NHS ester and converting amine to thiol and then use maleimide chemistry, will produce random biotin on the antibody. In some cases, labeling in some Fab areas could block antigens binding. With site specific biotinylation chemistry, biotin linkers are inserted to thiol-bridge locations and do not affect antibody-antigen binding (Fig.1). It will help increase the sensitivity of bio assay such as ELISA, Western Blot, etc.



Biotin Linker Mol. Wt.: 1182.466

Fig. 1 site-specific biotinylation of antibody

This conjugation reaction is simple, site-specific, and efficient. These kits include biotin linker, reaction buffer, protein concentrator, DMSO, and TCEP. The user can follow easy protocol and get the conjugation in less than 2 hours.

These kits are specifically optimized to label antibodies at scales of 100 µg and 1 mg. The kits are packaged as convenient single-use microtubes, eliminating difficulties associated with weighing small quantities of reagent.

Technical considerations

Prepare antibody solution at a preferred concentration of 2 mg/ml.

Note

- If you have a different concentration, adjust the antibody concentration accordingly.
- The antibody should be dissolved in reaction buffer pH 8.0 provided or 1X phosphate buffered saline (PBS), pH 8.0 with 1mM EDTA. If the antibody is dissolved in glycine buffer, it must be dialyzed against 1X PBS, pH 8.0 with EDTA, 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 a final antibody concentration range of 1-5 mg/ml is recommended.

Conjugation Experimental Protocol

1. Add antibody stock to a vial of TCEP, mix well, and incubate at 37 °C for 1 hour.
2. Reconstitute a vial of Biotin Linker (component A):
   a. For 100 µg kit, add DMSO not to be more than 10% of total reaction. For example, 2 µl of DMSO if the antibody concentration is 5 mg/mL, add up to 5 µl for concentration is 2 mg/mL, mix to dissolve.
   b. For 1 mg kit, add 10 µl of DMSO, mix to dissolve.
3. After incubation of antibody mixture from step 1 above, add all of the antibody to the vial of reconstituted biotin linker, and mix. Incubate with rotation, at room temperature for 1 hour.
4. Remove excess biotin reagent with protein concentrator by following the instruction for desalt.

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 1 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 – 10 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 – 10 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 the filter device, add to the microcentrifuge tube from step 5, and 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 (Cat# BP-50060) to calculate the degree of labeling (DOL).

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 thiol groups	1. If buffer contains thiol groups, buffer exchange the antibody into a non-thiol containing buffer such as the reaction buffer provided, using protein concentrator or 1x PBS pH8.0 with EDTA by dialysis. 2. Use TCEP reducing agent provided with the kits. If end-users provide their own reducing agent and use DTT, then it needs to be removed prior to the addition of biotin linker.
	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.