MagicLink™ Site-Specific Streptavidin Antibody Conjugation Kit

Components

Components		Product size			Storage condition
		BP-50XXX	BP-50XXX	BP-50XXX	
		1 x 100 µg	3 x 100 µg	3 x 500 µg	
A	MAGIC Site-Specific Linker (MW ~1,100)	1 vials	3 vials	3 vials	-20°C
B	LINK activated Streptavidin	1 vial	3 vials	3 vials	-20°C
C	Link 650 dye (BP-50066)	1 vial	1 vial	1 vial	-20°C
D	TCEP HCl	1 vials	3 vials	3 vials	-20°C
F	Protein Concentrator	10K MWCO (1)	10k MWCO (3)	10K MWCO (3)	RT
G	Reaction Buffer	15 ml	30 ml	30 ml	4-8 °C

Overview

MAGICLINK™ Site Specific Kits provide optimized reagents for labeling antibodies. The most important features of the kits are ultra-fast reaction speed, high conjugation efficiency, and the most stable linkage between antibody and streptavidin on the market to date. Labeling reactions chemiselectively target disulfide bonds for maximum labeling efficiency.

The kits provide optimized reagents for conjugating LINK modified streptavidin to antibodies or other proteins with disulfide bonds. Each 100 µg kit reaction is sufficient for labeling 50-200 µg of antibody in 100 µL reaction volumes, and 500 µg kit for 500 - 1000 µg antibody. The MAGIC Site-Specific linker, provided as a controlled excess reagent, reacts with the antibody under mild alkaline pH conditions. The MAGIC-antibody then reacts instantly with LINK-Streptavidin to get the Streptavidin-Antibody conjugate. Additionally, the kits include LINK-650 dye to determine the number of MAGIC molecules on the activated antibody.

Current antibody labeling methods, such as amidation or amine/thiol conversion for maleimide chemistry, produce random labels on the antibody. In some cases, labeling in Fab areas could block antigen binding. With site-specific cysteine/disulfide chemistry, these linkers are inserted to disulfide bonds and do not affect antibody-antigen binding (Fig.1). It will help increase the sensitivity of bio assay such as ELISA, Western Blot, etc.

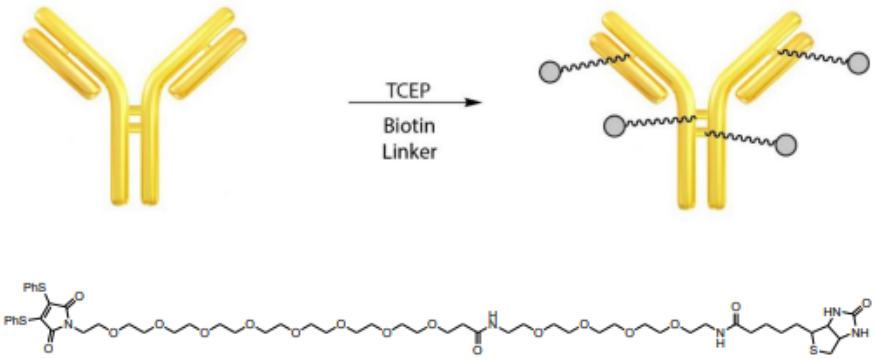
As expected for any chemical conjugation reaction, the concentration and buffer formulation of the Link-Streptavidin and the antibody need to fall within certain parameters, as detailed in this user guide.

Technical Considerations

1. Pre-conjugation considerations for the **Antibody**

   For optimal result, the antibody must be purified and have a final concentration between 1 – 10 mg/ml in reaction buffer, or 1X PBS pH 8.4-9.0 (see below for additional information). Ideally, 100 µl of antibody would react with 100 µl of Link-Streptavidin.

   Note
   - If you have a different concentration, adjust the antibody volume accordingly to make ~100 µg antibody



Biotin Linker Mol. Wt.: 1182.466

Fig. 1 site-specific biotinylation of antibody

   for 100 µg kit, and ~500 µg for 500 µg kit.
   - The antibody should be dissolved in 1X PBS, pH 8.4-9.0. If the antibody is dissolved in glycine buffer, it must be dialyzed against 1X PBS, or use Amicon Ultra-0.5, 10 kDa molecular weight cutoff (MWCO) for desalting (Cat # UFC501008 from Millipore Sigma) to buffer exchange into reaction buffer. The Amicon concentrator, can also be used 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 and should be removed accordingly.
   - The conjugation efficiency would be significantly reduced if the antibody concentration is less than 1 mg/ml. For optimal efficiency, a final antibody concentration range of 1-10 mg/ml is recommended.

2. Protein needs at least one cysteine available for conjugation. Disulfide-bridged cysteines work well too.
3. Protocol uses mild denaturing conditions via organic solvent.

Antibody Activation

Note:

1. For antibodies, the maximum number of MAGIC functional groups per molecule is 4, and is adjustable by changing the ratio of linker added. We recommend 2 equivalents of linker for each molecule added – 2 equivalents to get one molecule, 4 to get two molecules, etc.
2. The ratio of MAGIC functional groups per antibody can be measured by the provided Link-650 dye provided in this kit.
3. The kit also can be applied with other proteins besides antibodies. It is recommended to use 2 equivalents of linker per cysteine (including those in disulfides).

   [insert chart here testing different linker/Ab ratios maybe including SDS-PAGE]

1. Add entire antibody stock to TCEP vial and incubate at 37°C for 1 hour.
2. Use a protein concentrator to remove excess TCEP from the antibody solution, then reconstitute the antibody to 25 µM.
3. Reconstitute a vial of Magic Site Specific Linker to 10 mM using the DMSO provided.
   a. Adjust concentration of DMSO to 10-20% v/v.
   b. To dissolve 100 µg, need 10 µL of DMSO.
4. Add the appropriate amount of Magic Site Specific Linker to the reaction mixture, incubate at 4°C overnight with shaking (needs to be overnight? Can 1-2 hrs work?).
5. Use a protein concentrator to remove excess Magic Site-Specific linker from the antibody solution, then reconstitute the antibody with 300 µL reaction buffer. Repeat three times.

Generation of Antibody-Streptavidin Conjugate

This kit can be used to generate antibody-streptavidin conjugates with a range of antibody to streptavidin ratios. Simply add different amounts of streptavidin to the antibody, as described in the table below. The preferred ratio will depend upon the experiment that the conjugate will be used in, and may need to be determined experimentally. For comparison, the SDS-PAGE gel image in the analysis of the antibody-streptavidin conjugate section represents conjugates of different ratios of antibody to streptavidin.

1. Solvate the LINK activated Streptavidin in 50 µl DI H2O and mix by pipetting or by vortex.
2. Use all 50 µl of LINK-streptavidin to react with 100 µg (100 µg kit application), or 1 mg (1 mg kit application) MAGIC-antibody (generally 150 kDa) at 5x streptavidin to antibody mole ratio. Use 40 µl of LINK-streptavidin for 4x ratio, 20 µL for 2x, etc. Incubate at room temperature for 1 hour, preferably with rotation.
3. Your conjugate is now ready for use. You may also purify the conjugate to remove any unbound streptavidin if this is required for your application, use Amicon Ultra-0.5 30K MWCO provided in kit. Higher purity streptavidin-antibody conjugates can be obtained by ion-exchange chromatography (IEX) or SEC if needed.
4. Any unused activated streptavidin should be stored at - 20°C.

De-salting Procedure

Note: this step is to remove excess reagents and other side products at multiple points in the protocol.

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 minute. Discard liquid from filter device and collection tube.
2. Spin down by adding the labeled antibody to the concentrator/filter device up to 500 µl. Microcentrifuge at 14,000 x g for 8-10 minutes, or to minimum volume (~ 50 µl) 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 labeled 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.

Storage

Long-term stability of the antibody-streptavidin conjugate will depend on many factors, including the antibody and streptavidin themselves, the storage temperature, and storage conditions. In order to maximize stability, we would recommend storing the conjugate in a form that is as concentrated as possible, and at a low temperature. We would suggest checking with the antibody manufacturer if their products can be stored in 50% glycerol at -20°C. You should be able to store most conjugates in this condition, which would be compatible with the unconjugated antibody, and LINK streptavidin as well. If it is appropriate for your reagents and subsequent experiments, the addition of preservatives may also be helpful.

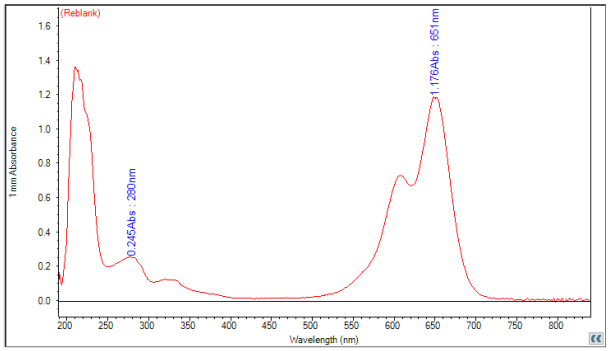
Calculation of the number of MAGIC groups/antibody ratio

Conjugation with Link-650 dye

1. Mix the Magic -protein conjugate with a small amount of the Link-650 dye and incubate with shaking for 1 hour.
2. Desalt 3 times using a 10K MWCO, or repeat until the flowthrough is no longer dark blue.
3. Test the UV/Vis absorbance at 280 and 650 nm.
4. Calculate degree of substitution using the following equation:
    a. A_{648} = absorption at 648 nm
    b. ε_{Ab} = extinction coefficient for IgG antibodies i.e. 210,000
    c. A_{280} = absorption at 280 nm
    d. ε_{dye} = extinction coefficient of the dye i.e. 270,000

$$\frac{Magic}{Protein} = \frac{A_{648} * \varepsilon_{protein}}{(A_{280} - 0.03 * A_{648}) * \varepsilon_{dye}}$$

Dye	Abs Max	Emi. Max.	Ɛ(dye)	CF_{280}
650-LINK	648	668	270000	0.03



Analysis of the Antibody-Streptavidin Conjugate

The antibody-streptavidin conjugates can be easily confirmed by gel electrophoresis or by conjugation with a Link-650 dye for example. Other methods, such as DNA gel or MALDI-TOF MS can also be used.

SDS-PAGE Analysis

1. Mix the conjugate sample (2 - 3 µg) with gel reducing buffer (not supplied) and heat at 100°C for 2 minutes.
2. Cool the sample, then load onto a SDS gel. A 4-12% gradient gel is recommended for best results.
3. Stain for protein using Coomassie Blue stain or a suitable equivalent. After destaining, the gel can be analyzed for the presence of antibody-streptavidin conjugates.

Troubleshooting

Problem	Possible Cause	Solution
Low or no labeling reaction	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 pH 8.0 with EDTA by dialysis. 2. Use TCEP reducing agent provided with the kits. If end-users provide their own reducing agent and
	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.
	MAGIC NHS, LINK NHS hydrolyzed	Use reagent immediately upon reconstitution
	Carrier protein was present in the antibody solution	Remove carrier protein before each conjugation by using Protein A, G or A/G resin or an antibody clean-up kit, this will reduce competition for the conjugation reaction