MagicLink™ Site Specific Oligo-Antibody Conjugation Kit

Components

Components		Product size			Storage condition
		BP-50144	BP-50145	BP-50146	
		1 x 100 µg	3 x 100 µg	1 x 1 mg	
A	Magic Site-Specific Linker (MW ~1,100)	2 vials*	4 vials**	2 vials*	-20°C
B	Link NHS (MW ~900)	2 vials*	4 vials**	2 vials*	-20°C
C	Link 650 dye (BP-50066)	1 vial	1 vial	1 vial	-20°C
D	TCEP HCl	2 vials*	4 vials**	2 vials*	-20°C
E	DMSO	1 mL	1 mL	1 mL	RT
F	Protein Concentrator	3K MWCO (2) 10K MWCO (2)	3K MWCO (4) 10k MWCO (4)	3K MWCO (2) 10K MWCO (2)	RT
G	Reaction Buffer	10 ml	30 ml	30 ml	4-8°C

Note: The kit above is designed for IgG antibodies, but works well for any disulfide-containing biomolecule. Please follow same technical tips if required

* 1 reaction + 1 control; ** 3 reactions + 1 control

Overview

MagicLink™ Site Specific Conjugation Kits provide optimized reagents for crosslinking antibodies via their interchain disulfide bonds. The target antibody is first reduced before activating with the MagicLink™ Site-Specific linker. Separately, the oligonucleotide is labeled through amine-NHS labelling. Finally, the two are mixed together for an instantaneous reaction, yielding the final conjugate. Through the use of click chemistry, this procedure is simple, site-specific and high yielding.

The benefit of this linker chemistry compared to traditional thiol-reactive functional groups such as maleimide or haloacetamides is the ability to rebuild disulfide bonds following reduction. One linker molecule may react with two cysteines, thereby preserving native protein structure.

The materials included in this kit are designed to label antibodies at scales of 100 µg and 1 mg. Each reagent comes pre-packaged for one-time use, eliminating difficulties associated with weighing small quantities of reagent. This protocol is simplified to reduce complexity for the user. For more detailed reaction considerations, please look to the end of this document.

Simplified Protocol

1. Transfer the stock solution of the antibody to a vial of TCEP HCl and incubate at 37°C for 30 minutes.
2. Dissolve the Magic Site-Specific linker with a small amount of DMSO. Transfer this solution to the antibody solution. Leave reacting at room temperature for >1 hour.
3. Dissolve the Link-NHS with a small amount of DMSO. Transfer this solution to the amine-labeled oligonucleotide. Leave reacting at room temperature for >1 hour.
4. Filter out unreacted Magic and Link-NHS from the two reaction mixtures using MWCO filters.
    a. Pre-wash the membrane by spinning with ~400 µL of DI water for 3 mins at 14,000 x g. Discard solution in upper and lower chambers.
    b. Dilute the reaction mixture with buffer until the DMSO concentration is below 5% v/v.
    c. Transfer the reaction mixture to the MWCO filter.
    d. Spin for 15 mins at 14,000 x g. Discard solution in lower chamber.
    e. Dilute solution in upper chamber with buffer.
    f. Repeat steps d-e twice.
    g. Transfer the solution from the upper chamber to fresh vial for final conjugation.
    h. (Optional) Wash vial with DI water to maximize recovery.
    i. Transfer the wash solution to the filtered solution (recovered in step g).
5. React the Magic-antibody and Link-oligo by mixing them together in a fresh vial. Leave reacting for >1 hour.
6. (Optional) Analyze results by SDS-PAGE or other method of choice.

Technical considerations

Pre-conjugation considerations for the **antibody**

The protein and oligo should both be pure, with a concentration of 2 mg/ml in 1xPBS, pH 7.4-8.4, or the kit's reaction buffer. If the solution contains glycine, then it must be buffer exchanged for 1xPBS, pH 7.4-8.4. This can be done through dialysis or by using MWCO filters such as those provided in the kit. This process removes small molecules as well as salts used in antibody precipitation. The solution must also be free of larger proteins such as BSA or gelatin.

Pre-conjugation considerations for the **amine-modified oligo**

This kit can be used to conjugate both amine-modified single and double-stranded oligos. A single-stranded oligo should be 20-120 bases long, while double stranded oligos can be up to 80 bp long. The final oligo needs to include an amine -CH_2NH_2 group added during synthesis. Most commercial oligo suppliers offer this modification. The amine position can be 5', 3' or 2' on the ribose. The oligo should also be at a concentration less than 100 µM for optimal reactivity.

Finally, the amine needs to be in its 'free base' form rather than as an $-NH_3^+$ salt. To guarantee this, dilute the amine-labeled oligo with 1N NaOH, then concentrate the solution using an MWCO filter. The final solution should be ~100 µl of an aqueous phosphate buffer with pH 7-8.

Experimental Protocol (for 100µg and 1mg kit)

1. Thiol-Reactive Labeling (100 µg or 1 mg kit)

Transfer the target antibody stock to a vial of TCEP HCl and leave reacting for 30 minutes at 37°C. Dissolve the Magic Site-Specific Linker (Component A) with a small amount of DMSO, such that the final reaction mixture is below 20% DMSO (v/v). Ensure it dissolved well before proceeding. Then transfer the entirety of the Component A stock solution to the protein solution. For the 100 µg kit, this can be up to 1 mg antibody, and for the 500 µg kit, this is up to 5 mg. Mix well by pipetting or vortexing. Let the reaction proceed at room temperature for at least 1 hour. Then proceed to purification with the MWCO filter. Be sure to use the activated protein within ~2 hours of purification.

2. Oligo Labeling (100 µg or 1 mg kit)

Dissolve Magic-NHS with DMSO: 50 µl for the 100 µg kit or 250 µl for the 500 µg kit, for a target concentration of 2 mg/ml. Add 100 µl of the dilute (< 100 µM) oligo solution to a vial of Component A, Magic-NHS. Mix well by pipetting or vortexing. Let the reaction proceed at room temperature for at least 1 hour. Continue onto Antibody Activation while the reaction is in progress.

3. Purification of Activated Proteins using MWCO

The purification procedure is the same for both conjugates.

a. Pre-wash the membrane by spinning with ~400 µL of DI water for 3 mins at 14,000 x g. Discard solution in upper and lower chambers.
b. Dilute the reaction mixture with buffer until the DMSO concentration is below 5% v/v.
c. Transfer the reaction mixture to the MWCO filter.
d. Spin for 15 mins at 14,000 x g. Discard solution in lower chamber.
e. Dilute solution in upper chamber with buffer.
f. Repeat steps d-e twice.
g. Transfer the solution from the upper chamber to fresh vial for final conjugation.
h. (Optional) Wash vial with DI water to maximize recovery.
i. Transfer the wash solution to the filtered solution (recovered in step g).
j. Take note of the final volume of the conjugate solution. Ideally, this is under 300 µl.

4. Generation of the Final Conjugate

To make the final conjugate, simply mix both filtered solutions in a vial. The reaction is instantaneous, occurring within minutes. Leave the reaction to proceed for at least 1 hour.

The final solution will contain a mixture of antibodies with different numbers of labels, as well as some unlabeled proteins. This is due to varying number of cysteine residues per protein, and the availability of multiple cysteines to react with a given linker molecule. IgG1 for example may conjugate four linkers, one per disulfide bond. These problems cannot be avoided, and the removal of these large molecule impurities is outside the scope of this procedure.

Storage

We recommend storing the conjugate at -20°C and at a high concentration. The optimal storage buffer is 50% glycerol/water. Preservatives like sodium azide are also tolerated.

Analysis by SDS-PAGE

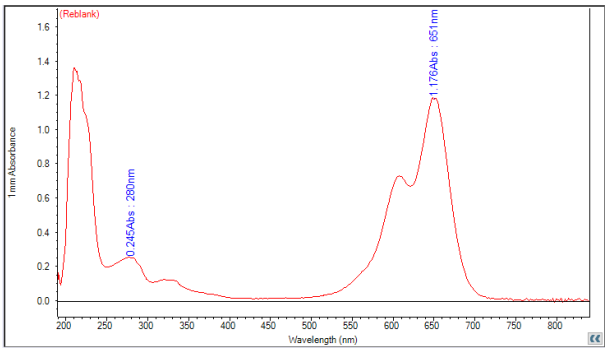
The simplest way to confirm conjugation is through SDS-PAGE gel electrophoresis. More specifically, a small amount (2-3 μg) of the conjugate can be run on a reducing SDS-PAGE gel.

1. Mix the conjugate sample 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-oligo conjugates. A typical gel image of control IgG-oligo conjugates is shown in Fig. 2.

Calculating the Labeling Efficiency using Link-650 (BP-22424)

1. Mix the Magic-Antibody conjugate with a small amount of the Link-650 dye and incubate with shaking for 1 hour.
2. Desalt at least 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:

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



Where:
A_{648}: the conjugate absorption at 648nm
E_{Ab} : extinction coefficient of Ab at 280 nm i.e. 210,000 $M^{-1}cm^{-1}$
A_{280}: the conjugation absorption at 280 nm
ε_{dye}: extinction coefficient of the dye i.e. 270000 $M^{-1}cm^{-1}$

Reaction Details

Magic-Antibody Disulfide Conjugation (example)

Component	MW	Mass (µg)	nmol	Ratio	Volume (µL)	Initial Conc.	Final Conc.
TCEP HCl	250	16	63.9	Excess			
Magic Site-Specific Linker (in DMSO)	1083	100	92.3	13.8	50	2.0 mg/ml	0.18 mg/ml
IgG1 (aq.)	150,000	1000	6.67	1	500	2.0 mg/ml	1.8 mg/ml
DMSO						100%	20%

Link-Oligo Conjugation (example)

Component	MW	Mass (µg)	nmol	Ratio	Volume (µL)	Initial Conc.	Final Conc.
Link-NHS (in DMSO)	867	100	115.34	10	50	2.0 mg/ml	0.6 mg/ml
50-mer ssDNA Oligo (aq.)	20,000	230.6	11.53	1	100	2.3 mg/ml	1.5 mg/ml
DMSO						100%	33%

Antibody-Oligo Conjugation (example)

Component	MW	# Linkers	Mass (μg)	nmol	Ratio
Magic-Oligo	20,000	1 amine	106	5.33	8.0
IgG1 mAb	150,000	4 disulfides	100	0.66	1.0

Troubleshooting

Problem	Possible cause	Solution
NHS Labeling: Low or no reaction	Buffer containing primary amine	Buffer exchange the antibody into a non-amine-containing buffer such as the reaction buffer provided. This can be done by dialysis or by MWCO filtration.
	Link-NHS hydrolyzed	Use reagent immediately upon reconstitution, or raise the concentration of the target protein
	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
	Amine modified oligo, amine as salt form	Desalt with 1N NaOH, and then equilibrate back with the reaction buffer.
Thiol Labeling: Low or no reaction	Buffer containing thiol groups	Buffer exchange the antibody into a non-thiol-containing buffer such as the reaction buffer provided. This can be done by dialysis or by MWCO filtration.
	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