MagicLink™ Oligonucleotide Antibody Conjugation Kit

Components

Components		Product size			Storage condition
		BP-50005	BP-50004	BP-50003	
		1 x 100 µg	3 x 100 µg	1 x 1 mg	
A	MAGIC NHS (MW ~900)	1 vial	3 vials	1 vial	-20°C
B	LINK NHS (MW ~900)	1 vial	3 vials	1 vial	-20°C
E	Reaction Buffer	10 ml	30 ml	30 ml	4-8 °C
F	Protein Concentrator	3K MWCO (1) 10K MWCO (1)	3K MWCO (3) 10k MWCO (3)	3K MWCO (1) 10K MWCO (1)	Room temperature

Note: The kit above is designed for IgG antibodies, but works well for any amine containing biomolecule.

Overview

BroadPharm's MagicLink system is tailor-made to produce oligonucleotide-antibody conjugates without the headache. The resulting conjugates are stable, water-soluble, and easily quantified. The procedure is simple, takes less than 2 hours, and avoids denaturants or reducing conditions.

The kits provide optimized reagents for conjugating amine modified oligonucleotides to IgG antibodies. Using Magic-NHS, each 100 µg kit can conjugate up to 1 mg IgG antibody. Similarly, the Link-NHS ester reacts with amine-labeled oligonucleotides. Centrifugal filtration removes excess Magic and Link-NHS reagents. Finally, mixing the labeled antibody and oligo together forms the final conjugate within seconds.

This kit is ideal for IgG antibodies and amine-labeled oligonucleotides. Solid phase oligonucleotide manufacturers provide this modification at a cost-efficient solution. Single stranded oligos should be 10-120 bp while double-stranded oligos can be up to 80 bases long.

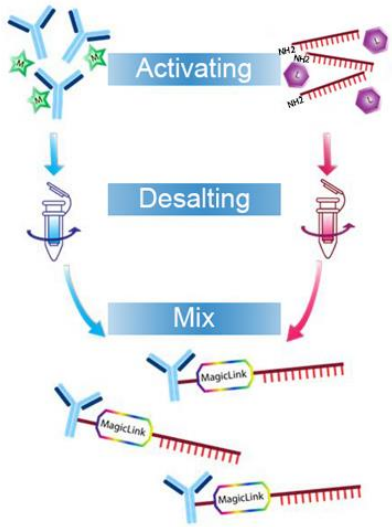

Figure 1 Schematic representation of the process of MagicLink™ Oligo-Antibody conjugation.

Simplified Protocol

1. Dissolve Magic-NHS with a small amount of DMSO. Transfer this solution to the oligo-containing solution. Leave reacting >1 hour.
2. Dissolve Link-NHS with a small amount of DMSO. Transfer this solution to the antibody-containing solution. Leave reacting >1 hour.
3. Filter out unreacted Magic- or Link-NHS 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 reaction buffer.
    f. Repeat steps d-e twice.
    g. Transfer the solution in the upper chamber to fresh vial for final conjugation.
    h. (Optional) Wash vial with DI water to maximize recovery. Transfer solution for final conjugation.
4. React the activated oligo and antibody by mixing them together in a fresh vial. Leave reacting for >1 hour.
5. (Optional) Remove excess oligo by using a 50K MWCO, following the same procedure as in Step 3.
6. (Optional) Analyze results by SDS-PAGE or other method of choice.

Technical considerations

1. 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. All 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 unreactive -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.

2. Pre-conjugation considerations for the **antibody**

The antibody should be pure, with a concentration of 2 mg/ml, preferably in the kit's reaction buffer. If the solution contains glycine, then it must be buffer exchanged for 1xPBS, pH 7.2-7.4. 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.

Experimental Protocol

1. Oligo Labeling (100 µg or 500 µg 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.

2. Antibody Activation

Dissolve Link-NHS 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 Link-NHS stock solution to the antibody 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 antibody and oligo within ~2 hours of purification.

Purification of Activated Oligo, and Antibody using MWCO

The purification procedure is the same for both conjugates, despite needing different MWCO filters. Be sure to use the 3K MWCO with the oligo, and the 10K MWCO with the antibody.

1. 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.
2. Dilute the reaction mixture with reaction buffer until the DMSO concentration is below 5% v/v.
3. Transfer the reaction mixture from oligo/antibody activation steps. Centrifuge for 14,000 x g for 10-15 minutes. Discard waste from the lower chamber. Add ~200 µl of reaction buffer to the upper chamber.
4. Repeat centrifugation/dilution procedure twice, for a total of three rounds of centrifugation.
5. Transfer the upper chamber's solution to a fresh vial for final antibody-oligo conjugation.
6. (Optional) Transfer 100 µl of DI water into the same MWCO filter and transfer this solution to the fresh vial as well
7. Take note of the final volume of the conjugate solution. Ideally, this is under 300 µl.

Generation of Antibody-Oligo Conjugate

To make the final conjugate, transfer the desired amount of oligo to the activated antibody. Leave the reaction to proceed for at least 1 hour. After, the solution can be filtered again with MWCO's to remove unreacted oligos, if so desired.

This kit can generate conjugates with upwards of 15 oligonucleotides per antibody. Note that this number is not fixed and will vary between different antibodies. Also note that this ratio is only an average, as not every antibody will have the same number of oligos. Adjust this ratio by adding only a fraction of the activated oligo to the antibody solution. The preferred ratio ultimately depends on the downstream applications for the conjugate.

The exact volume will depend on the amount of oligo and the total volume of the purified solution. For example, 20 nmol (150 µg) of a 20-mer dsDNA oligo with 500 µg antibody can make a conjugate with 6 oligos per antibody. The user would add the full stock of activated oligo to the antibody solution. But if the user wanted a conjugate with 3 oligos per antibody, they could add only half of the total volume instead.

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 of the Antibody-Oligo Conjugate

The simplest way to confirm conjugation is through SDS-PAGE gel electrophoresis. Other methods like mass spectrometry are more accurate in determining antibody-oligo ratios.

Example Protocol for SDS-PAGE:

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.

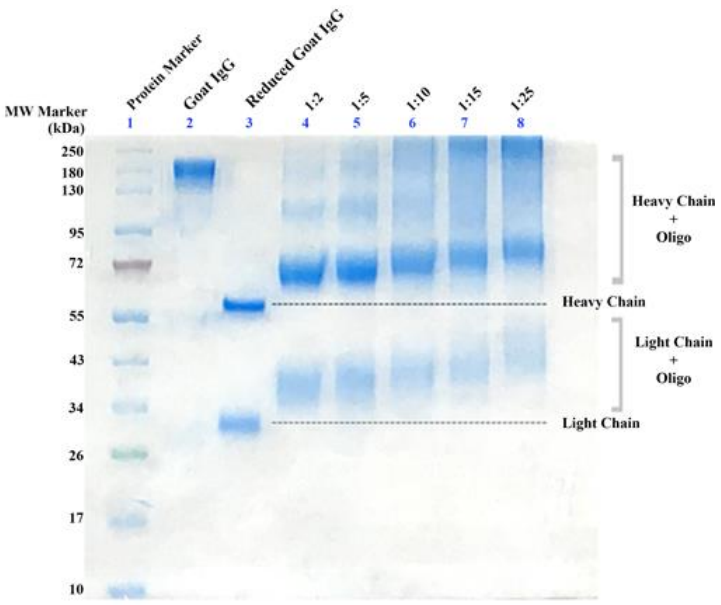

Fig 2. Gel image of oligonucleotide-antibody conjugates A 4-12% bis-tris gel confirming conjugation between a goat IgG (control) and the oligo (control) prepared by MagicLink ™ oligo antibody conjugation kit. Five different antibodies: oligo ratios with 3 μg of conjugate loading.

Reaction Details

Note: This table is made for a hypothetical Oligo-Antibody conjugation procedure. The numbers should be adjusted based on the molecular weight of the target oligo, amount of antibody/oligo, the specific kit used, and so on.

Magic-Oligo Conjugation (example)

Component	MW	Mass (μg)	nmol	Ratio	Volume (μL)	Initial Conc.	Final Conc.
Magic-NHS (in DMSO)	886	100	115.34	2.3	50	2.0 mg/ml	0.7 mg/ml
50-mer ssDNA Oligo (aq.)	16,500	825	50.00	1	100	8.3 mg/ml	5.5 mg/ml
DMSO						100%	33%

Link-Antibody Conjugation (example)

Component	MW	Mass (μg)	nmol	Ratio	Volume (μL)	Initial Conc.	Final Conc.
Link-NHS (in DMSO)	867	100	115.34	17.3	50	2.0 mg/ml	0.4 mg/ml
IgG1 Antibody (aq.)	150,000	1000	6.67	1	500	2.5 mg/ml	2.0 mg/ml
DMSO						100%	20%

Troubleshooting

Problem	Possible cause	Solution
Low or no reaction	Buffer containing primary amine	Buffer exchange the antibody into a non-amine-containing buffer such as reaction buffer provided, or PBS by desalting columns or dialysis
	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
	Amine modified oligo, amine as salt form	Desalt with 1N NaOH, and then equilibrate back with the reaction buffer.