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	3 x 500 µg	
A	MAGIC NHS (MW ~900)	2 vials*	4 vials**	4 vials**	-20°C
B	LINK NHS (MW ~900)	2 vials*	4 vials**	4 vials**	-20°C
C	Oligo Control	1	1	1	-20°C
D	Antibody Control	1	1	1	-20°C
E	Protein Concentrator	3K MWCO (2) 10K MWCO (2)	3K MWCO (4) 10k MWCO (4)	3K MWCO (4) 10K MWCO (4)	RT
F	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 amine containing biomolecule. Please follow same technical tips if required

* 1 rxn + 1 control; ** 3 rxn + 1 control

Overview

The Oligonucleotide-Antibody Conjugation Kits make use of BroadPharm's MagicLink™ technology to produce oligo-antibody conjugates. The most important features of the kits are ultra-fast reaction speed, high conjugation efficiency, and the most stable linkage between antibody and oligonucleotide on the market to date. This crosslinking reaction does not need reducing agent such as DTT, TCEP, or catalysts. The kits generate antibody-oligo conjugates in less than 2 hours.

The kits provide optimized reagents for conjugating amine modified oligonucleotide to antibodies. 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 NHS ester, provided as a controlled excess reagent, reacts with the amine modified oligonucleotide under mild alkaline pH condition. Similarly, the LINK ester will react with antibody under the same condition. Prior to coupling, LINK-antibody and MAGIC-oligonucleotide are desalted with centrifugal concentrators. The LINK-antibody then reacts instantly with MAGIC-oligonucleotide to get the oligonucleotide-antibody conjugates. Additionally, the kits include positive controls for confirmation of conjugation chemistry.

These kits can be used to conjugate antibodies to single-stranded oligos that are 10-120 bases long or to double-stranded oligos that are up to 80 bases long. The oligos is amine-modified one which can be done in a regular Oligo synthesis lab.

The kits are available in three formats.
The 1-test kit includes reagents for one conjugation of 100 µg of antibody and one control. The 3-test kits include reagents for three conjugations of 100 µg or 500 µg of antibody and one control.
As expected for any chemical conjugation reaction, the concentration and buffer formulation of the oligo and the antibody need to fall within certain parameters, as detailed in this user guide.

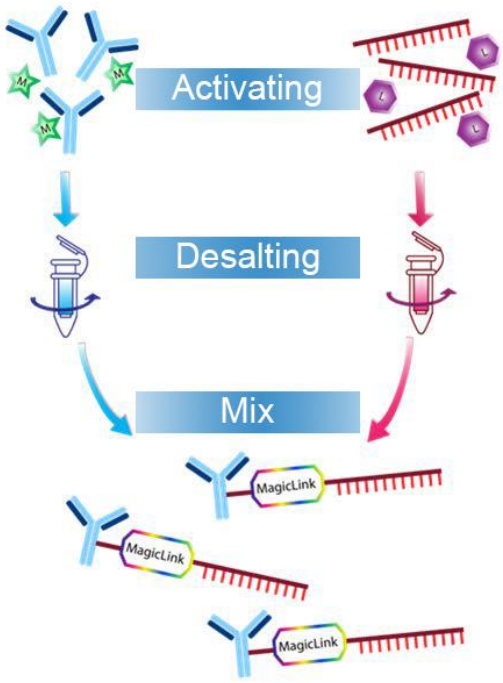

Figure 1 Schematic representation of the process of MagicLink™ Oligo antibody conjugation.

Technical considerations

1. This kit is provided with control reagents for a test trial before proceeding to the actual conjugation of interest materials. Since laboratory environment and instrument may be different, it is recommended that the customer do a test reaction with the provided the oligo, and antibody controls first, and verify successful conjugation by SDS-PAGE.
2. 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 and contain a terminal NH_2 group, which must be added during synthesis. (All commercial oligo suppliers offer this modification). The efficiency of conjugation is slightly higher with 5' aminated oligos. Double-stranded oligos can be up to 80 bases in length but only one end should be aminated.

   The amine-modified oligo must be purified by HPLC to a final concentration of 60-100 µM in 100 µl of a suitable buffer (see below). If the oligo concentration is greater than 100 µM, dilute to 100 µM with reaction buffer. In addition, amine-modified oligo should be amine form, not TFA salt form. TFA salt can be converted to its amine form by desalt column with 1N NaOH and equilibrate by the reaction buffer provided.
   The reaction volume should not deviate much from 100 µl.

3. 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 pH7.2-7.4 (see below for additional information). Ideally, 100 µl of antibody would react with 100 µl of oligo.

Note

- If you have a different concentration, adjust the antibody volume accordingly to make ~100 µg antibody for 100 µg kit, and ~500 µg for 500 µg kit.
- The antibody should be dissolved in 1X PBS, pH7.2-7.4. 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.

SAMPLE EXPERIMENTAL PROTOCOL

1. Oligo Activation (100 µg or 500 µg kit)
   Add 100 µl of the oligo to the one MAGIC NHS ester vial (component A). Mix gently and incubate for 1 hour at room temperature. Proceed to next section, purification, after the incubation.

   Note: The activated oligo or control oligo should be used right away or within 2 hours.

2. Antibody Activation
   - 100 µg kit: Add 100 µl of the antibody (at 1 mg/ml concentration) to the LINK NHS ester vial (component B). Mix gently and incubate for 1 hour at room temperature. Proceed to next section, purification, after the incubation.
   - 500 µg kit: Add 500 µg of antibody to LINK NHS ester vial (component B); i.e., 100 µl Ab (5 mg/ml). Mix gently and incubate for 1 hour at room temperature. Proceed to next section, purification, after the incubation.

   Note:
   - The activated antibody or control antibody should be used right away or within 2 hours.
   - For all kits, the control oligo and antibody are provided enough for 100 µg control antibody reaction (it mimics a 100 µg conjugation kit). Reconstitute control oligo (component C), and control antibody (component D), each in 100 µl of reaction buffer. Follow above instruction - sample experimental protocol; 1, and 2 (100 µg kit).

Purification of activated oligo and antibody

Note: For oligo desalting, use Ultra-0.5 3K MWCO concentrator, antibody desalting uses Ultra-0.5 10K MWCO concentrator.

1. Hydrate concentrator membrane 'filter devices' with 400 to 500 µl of reaction buffer or DI water, and microcentrifuge 14,000 x g, for 3 minutes. Discard liquid from filter devices and collection tubes.
2. Spin down by adding activated samples to the concentrators/filter devices up to 500 µl. Microcentrifuge at 14,000 x g, 8 - 10 minutes, or to minimum volume ~ 50 µl left in the filter devices. Discard waste from the collection tubes.

3. Desalt by adding reaction buffer to the filter devices up to 500 µl. Microcentrifuge at 14,000 x g, 8 - 10 minutes, or to minimum volume ~ 50 µl left in the filter devices. Discard waste from the collection tubes.
4. Repeat step 3, twice.
5. Transfer activated samples from the filter devices into separate microcentrifuge tubes.
6. Optional for maximum recovery, add reaction buffer, volume determined by the user, to the filter devices to rinse out residual samples, microcentrifuge pulse spin, collect antibody/reaction buffer from filter device, add to the microcentrifuge tube from step 5, mix. (Example, sample 1 mg/ml starting volume 100 µl (step 2) recovered 50 µl (step 5), add 50 µl to rinse (step 6), final volume 100 µl).
7. Optional determine sample concentrations to ensure recovery, then proceed to the next section, follow table.

Generation of Antibody-Oligo Conjugate

This kit can be used to generate antibody-oligo conjugates with a range of antibody to oligo ratios. Simply add different amounts of oligo 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 gel image in the analysis of the antibody-oligo conjugate section represents conjugates of different ratios of antibody to oligo.

1. Add 100 µl of activated antibody to the appropriate volume of activated oligo and 1X PBS in a microcentrifuge tube, as shown in the table below.

Volume of activated antibody (µL)	Volume of activated oligo(µL)	Volume of PBS Buffer (µL)	Antibody: oligo starting molar ratio
100 µL	100	0	1:15
100 µL	67	33	1:10
100 µL	33	67	1:5
100 µL	20	80	1:3

Note: The antibody: oligo ratio is an average since a population of labeled antibodies will be produced following the conjugation reaction. Each antibody will not have exactly the same number of oligos bound to it.

2. Mix and incubate at room temperature for 1 hour. Conjugations can also be incubated over night at room temperature with no adverse effect.
3. Your conjugate is now ready for use. You may also purify the conjugate to remove any unbound oligos if this is required for your application, use Amicon Ultra-0.5 100K MWCO (not provided), and follow manufacturer's instruction. High purify of the oligo-Ab conjugates can be performed by ion-exchange chromatography (IEX) if needed.
4. Any unused activated oligo should be stored at - 20°C.

Storage

Long-term stability of the antibody-oligo conjugate will depend on many factors, including the antibody and oligo 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 and oligo manufacturers 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 oligo as well. If it is appropriate for your reagents and subsequent experiments, the addition of preservatives may also be helpful.

Analysis of the Antibody-Oligo Conjugate

The antibody-oligo conjugates can be easily confirmed by gel electrophoresis. Other methods, such as DNA gel or MALDI-TOF MS can also be used.

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.

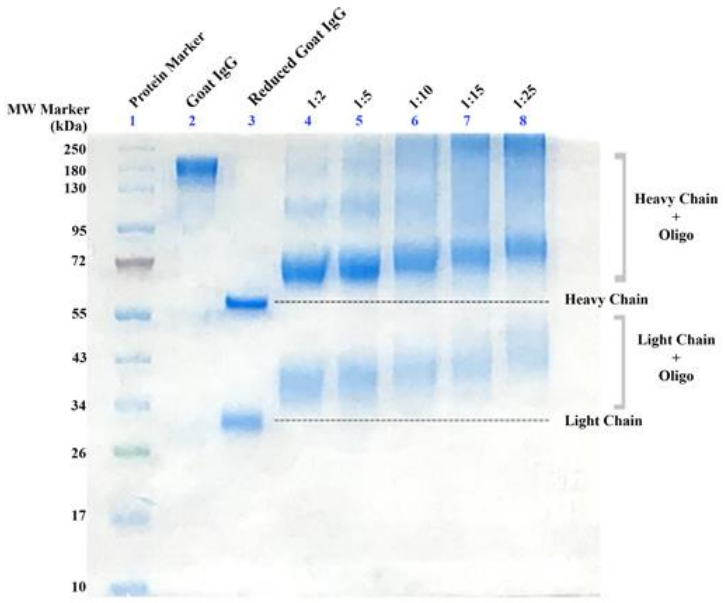

Fig 2. Gel image of oligonucleotide-antibody conjugates
A 4-12% bis-tris gel confirming conjugation between a goat IgG (control) and the oligo (control) prepared by MagicLink ™ oligo antibody conjugation kit. Five different antibodies: oligo ratios with 3 µg of conjugate loading.

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	Amine modified oligo as the TFA salt is most common form. Before labeling, the amine modified oligo should be free amine form, not salt form. Or desalt with 1N NaOH, and then equilibrate with the reaction buffer.