MagicLink™ HRP Antibody Labeling Kits

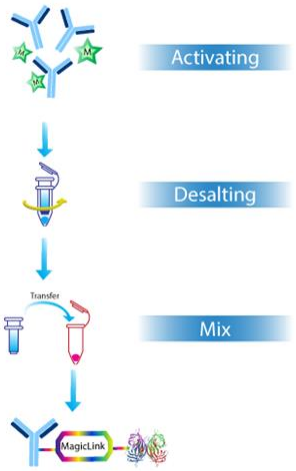
Components

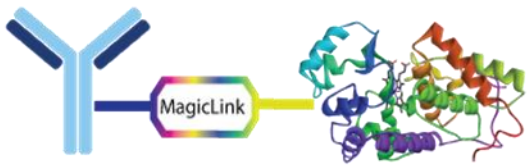
Components		Product size		Storage
		BP-50000	BP-50062	
		1 x 1 mg	5 x 1 mg	
A	MAGIC NHS (MW ~900)	1 vial	5 vials	-20C
B	LINK activated HRP	1 vial	5 vials	-20C
C	Reaction Buffer	10 ml	30 ml	RT
D	Protein concentrator	1	5	RT
Note: The kit above is designed for IgG antibodies, but works well for any amine containing biomolecule. Please follow same technical tips if required.				

Overview

MagicLink™ HRP Antibody Labeling Kits are the 3rd generation HRP conjugation technology which can be used to conjugate horseradish peroxidase (HRP) to protein, antibody, amine modified oligo, etc. The labeling kits feature the most stable linkage between HRP antibody on the market. The instant and efficient labeling reaction yield 95 to 100% HRP conjugates.

These kits are specifically optimized to label antibodies at a scale for 1 mg. The kits' format is based on instant reaction between functional group MAGIC and LINK at room temperature. By following the protocol provided in the kits, the end users can label their antibody with MAGJIC NHS to get MAGIC-antibody which instantly reacts with LINK activated HRP (provided in the kits) to achieve Ab-HRP conjugates.





At a Glance

Protocol summary

1. Add reaction buffer into antibody.
2. Transfer whole antibody solution to MAGIC NHS vial.
3. Incubate at room temperature for 60 minutes.
4. Remove excess MAGIC by concentrator/filtration device.
5. Mix MAGIC-antibody with LINK activated HRP.
6. Storage.

Note: Upon receipt, store the kits at 4°C. When stored properly, the kits should be stable for six months. Alternatively, components A and B can be stored at-20°C. Do not freeze reaction buffer (component C). Warm all the components and centrifuge the vials briefly before opening, and immediately prepare the required solutions before starting your conjugation. The following SOP is an example for labeling goat anti-mouse IgG antibody.

Preparation of Working Solution

For labeling 1 mg of purified antibody (assuming the target antibody concentration is 2 mg/ml), final volume would be in 500 µl of reaction buffer (component C). For lyophilized antibody, add 500 µl of reaction buffer to reconstitute. Antibody, in liquid form and not in 1X PBS, pH 7.2 – 7.5 may require buffer exchange, see note.

Note

- If you have a different concentration, adjust the antibody volume accordingly to make 1 mg antibody available for your labeling reaction.
- The antibody should be dissolved in 1X phosphate buffered saline (PBS), pH 7.5; if the antibody is dissolved in glycine buffer, it must be dialyzed against 1X PBS, pH 7.2-7.5, 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 the final antibody concentration range of 1-5 mg/ml is recommended.
- The presence of sodium azide will inhibit HRP activity.

Labeling Protocol

React Antibody with MAGIC NHS reaction:

Add the antibody solution directly into the vial of Magic NHS (component A), and mix them well by repeatedly pipetting for a few times or vortex the vial for a few seconds. Keep the antibody labeling reaction mixture at room temperature for 60 minutes. The antibody-labeling reaction mixture can be rotated or shaken for longer time if needed.

Purify the MAGIC-Antibody Solution

1. Hydrate concentrator membrane ‘filter device’ with 400 to 500 µl of 1X PBS or DI water, and microcentrifuge

14,000 x g, for 3 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 minutes, or to minimum volume ~ 50 µl left in the filter device. Discard waste from the collection tube.
3. Buffer exchange by adding 1X PBS to the filter device up to 500 µl. Microcentrifuge at 14,000 x g, 8 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 reaction buffer, volume determined by the user, to the filter device to rinse out residual antibody, microcentrifuge pulse spin, collect antibody/PBS from filter device, add to the microcentrifuge tube from step 5, mix.
7. Determine sample concentration, then proceed to the next section.

HRP-Antibody Conjugation

1. Make LINK-HRP solution by adding 50 µl ddH_2O into the vial of LINK-HRP (component B), mix well by repeatedly pipetting for a few times or vortex the vial for a few seconds.
2. Mix vial of LINK-HRP solution into the purified MAGIC-antibody solution (from step purify the MAGIC-antibody solution, above), at different ratios, mix well and rotating the mixture for 1 hour at room temperature.

Note:
- It is recommended the mix Link activated HRP and antibody at 3:1.
- Use all 50 µl of LINK-HRP to label 1 mg MAGIC-antibody (generally 150 kDa) at 5x HRP to antibody ratio mole ratio, 40 µL for 4x, 20 µL for 2x, etc.
- Magic activated protein/antibody should be used right away.
- For a different protein, user need optimize the Protein/HRP mix ratio for fit the application accordingly.

The HRP-antibody conjugate is now ready to use. For immediate use, the HRP-antibody conjugate need be diluted with the buffer of your choice. For longer term storage, HRP-antibody conjugate solution need be concentrated or freeze dried.

Storage of HRP-Antibody Conjugate

The antibody conjugate should be stored at > 0.5 mg/ml in the presence of a carrier antibody (e.g., 0.1% bovine serum albumin). For longer storage, the HRP-antibody conjugates could be lyophilized and stored at ≤ –20 °C.

Troubleshooting

Problem	Possible cause	solution
Low or no conjugation with MAGIC NHS	Buffer containing primary amine	Buffer exchange the antibody into a non-amine-containing buffer such as the PBS provided by desalting columns or dialysis
	MAGIC NHS was hydrolyzed	Use reagent immediately upon reconstitution
	Carrier protein was present the antibody solution	Remove carrier protein before conjugation by using Protein A, G or A/G resin or an antibody clean-up kit. This will reduce competition for labeling