MagicLink™ Alkaline Phosphatase Antibody Conjugation Kits

Components

Components		Product size			Storage condition
		BP-50090	BP-50091	BP-50092	
		1 x 100 µg	3 x 100 µg	1 x 1 mg	
A	MAGIC NHS (MW ~900)	1 vial	3 vials	1 vial	-20C
B	LINK activated AP	1 vial	3 vials	1 vial	-20C
C	Reaction Buffer	10 ml	30 ml	30 ml	RT
D	Protein Concentrator	N/A	N/A	1	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™ Alkaline Phosphatase (AP) Antibody Conjugation Kits are the 3rd generation AP conjugation technology which can be used to conjugate AP to protein, antibody, amine modified oligo, etc. The kits feature the most stable linkage between AP and antibody on the market. The instant and efficient reactions yield 95 to 100% AP conjugates.

These kits are specifically optimized to conjugate antibodies at a scale of 100 µg, and 1mg. The kits' format is based on instant reaction between functional groups MAGIC and LINK at room temperature. By following easy protocol provided in the kits, the end users can activate their antibody with MAGIC NHS to get MAGIC-antibody which instantly reacts with LINK activated alkaline phosphatase (provided in the kits) to achieve Ab-AP conjugates.

At a Glance

Protocol summary

1. Add reaction buffer to antibody.
2. Transfer whole antibody solution to MAGIC NHS vial.
3. Incubate at room temperature for 1 hour.
4. Remove excess MAGIC NHS by protein concentrator for 1 mg conjugation kit, 100 µg kit not required.
5. Mix MAGIC-antibody with LINK activated AP.
6. Incubate at room temperature for 1 hour.
7. Storage.

Note: Upon receipt, store the kit at 4°C. When stored properly, the kit 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 conjugating 100 µg antibody (assuming the target antibody concentration is 2 mg/mL), final volume would be in 50 µl reaction buffer (component C), or 1X PBS pH 7.2 – 7.5. For lyophilized antibody, add 50 µl of reaction buffer to reconstitute. Antibody, in liquid form and not in 1X PBS, pH 7.2 – 7.5 may require buffer exchange into reaction buffer, see note.

Note

- 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.
- Alternatively, use Amicon Ultra-0.5, 10 kDa cutoff (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, and buffer exchange into reaction buffer. It is necessary to use extra amount of starting antibody since some amount of antibody will be lost during buffer exchange.
- If you have a different concentration, adjust the antibody volume accordingly to make ~100 µg antibody available for your conjugation reaction.
- 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 efficiency the final antibody concentration range of 1-5 mg/ml is recommended.
- The presence of sodium azide will inhibit AP activity.

Antibody Activation Protocol

Antibody activation with MAGIC NHS:

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

Purify the MAGIC-Antibody/Protein Solution

Note: 100 µg kit desalting is not necessary, optional if user desires, concentrator not supplied with kit. Skip to next section, conjugation.

1. Hydrate concentrator membrane 'filter device' with 400 to 500 µl of reaction buffer 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 MAGIC labeled antibody/protein 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. Desalt by adding reaction buffer 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/reaction buffer from filter device, add to the microcentrifuge tube from step 5, mix.
7. Determine sample concentration, then proceed to the next section.

AP-Antibody Conjugation

1. Make LINK-activted AP solution by adding 50 µL ddH_2O into the vial of LINK-AP (component B), mix well by repeatedly pipetting for a few times or vortex the vial for a few seconds.
2. Mix LINK-AP solution with MAGIC-antibody solution at a desire ratio, mix well and rotate the mixture for 1 hour at room temperature.

Note:

- It is recommended to mix LINK-activated AP and antibody at a 1:1 ratio.
- Use all 50 µl of LINK-AP to label 100 µg of MAGIC-antibody (generally 150 kDa) at a 5x AP to antibody mole ratio, 40 µL of LINK-AP for 4x ratio, 20 µL for 2x, etc.
- Magic activated protein/antibody should be used right away.
- For a different protein, user need optimize the AP/Protein mix ratio for fit the application accordingly.

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

Storage of AP-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 AP-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 reaction buffer provided, or 1x PBS, using protein concentrator or dialysis tubing/cassette
	MAGIC NHS was hydrolyzed	Use reagent immediately upon reconstitution
	Carrier protein was present in 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 the conjugation reaction