MagicLink™ Streptavidin Labeling Kit

Components		Product size			Storage
		BP-50008	BP-50007	BP-50006	
		1 x 100 µg	3 x 100 µg	1 x 1 mg	
A	MAGIC NHS (MW ~900)	1	3	1	-20°C
B	LINK activated Streptavidin	1	3	1	-20°C
C	Reaction Buffer	15 ml	15 ml	15 ml	RT
D	Centrifuge Concentrator	N/A	N/A	1	RT

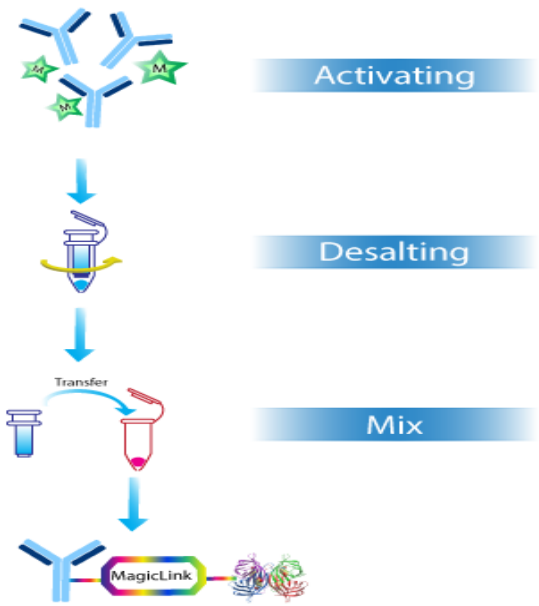
Overview

MagicLink Streptavidin Labeling Kit is a new generation of streptavidin labelling kit and it is featured as the most stable linkage between streptavidin and biopolymers on the market. The kit enables the instant conjugation of a pre-activated streptavidin to an biopolymers.

MagicLink Streptavidin Conjugation Kit can be used to label primary antibody, recombinant antibodies or fluorescent polymer such as BV 421, BV510, BV570, BV605, BV 650, BV 711, BV 785, PE, APC, or amine modified oligo, oligo- BV tandem dye, oligo-PE, oligo-APC conjugates, etc.

- High Conjugation efficiency around 95-100%.
- Instant reaction.
- Most stable covalent bonded conjugates on the market.
- Consistent from batch to batch.
- A wide range of target proteins – Also applicable to antibody fragments and small proteins.
- No DTT, TCEP, and reducing agents are needed.

This kit is optimized to label antibodies as an example at a scale for 100 µg and 1 mg. The kit format is based on an instant reaction between functional groups MAGIC and LINK at ambient temperature. By following the protocol provided, the end users can label their antibodies with MAGIC NHS to get MAGIC-antibodies that instantly react with LINK activated streptavidin (provided in the kit) to achieve Ab-streptavidin conjugates.



At a Glance

Protocol summary

1. Add reaction buffer to antibody.
2. Transfer antibody solution to MAGIC NHS working solution.
3. Incubate at room temperature for 60 minutes.
4. Exchange and remove excess MAGIC acid by centrifugal concentrator.
5. Prepare LINK activated streptavidin stock solution.
6. Mix MAGIC-antibody with LINK activated streptavidin.
7. Storage.

Note: Upon receipt, components A and B must 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

Pre-conjugation considerations for the protein.

This kit has been designed to conjugate 100 µg and 1 mg of antibody per reaction. For optimal results, the concentration should be adjusted to the concentration of 5 mg/ml in reaction buffer (see below). Higher protein concentrations should be diluted accordingly with reaction buffer.

Note:

- The antibody should be purified and amine, glycine, BSA, gelatin free. Glycine can be removed by dialyzing against 1X PBS, pH 7.2-7.4. Alternatively, use Amicon Ultra-0.5, Ultracel-10 Membrane, 10K MWCO (Cat # UFC501008 from Millipore). Impure protein or protein stabilized with bovine serum albumin (BSA) or gelatin will not be labeled well.
- For optimal labeling efficiency a final protein concentration range of 1-5 mg/ml is recommended. The conjugation efficiency is significantly reduced if protein concentration is less than 1 mg/ml.
- The presence of low concentrations of sodium azide (<3 mM) or thimerosal (<1 mM) will not interfere with the conjugation reaction.

Labeling protocol

Add antibody/protein solution to the MAGIC NHS vial. Mix well and incubate the reaction for 1 hour at room temperature with rotation. The labeled product is MAGIC-antibody.

Purify the MAGIC-Antibody/Protein Solution (1 mg kit)

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.

MAGIC LINK Streptavidin-Antibody Conjugation

1. Prepare LINK-streptavidin solution by adding 50 µl ddH2O into the vial of LINK streptavidin (component B), mix well by repeatedly pipetting for a few times or vortex the vial for a few seconds.

2. Add a whole vial of LINK-Streptavidin solution into the MAGIC-antibody solution, mix well and rotating the mixture for 1 hour at room temperature.

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

Note: For a different protein or other biomolecule, users need optimize the Protein/streptavidin mix ratio for fit the application accordingly.

Storage of Streptavidin -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 streptavidin-antibody conjugates could be lyophilized and stored at ≤ –20 °C.

Troubleshooting

Problem	Possible cause	solution
Low or no MAGIC conjugation	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 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 labeling