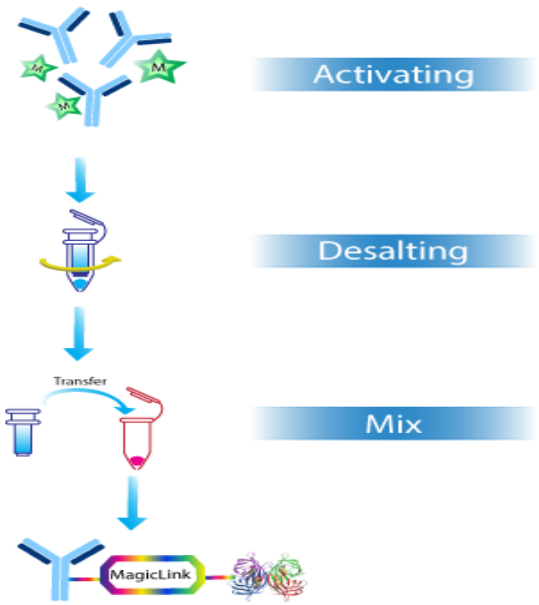
MagicLink™ Streptavidin Antibody Conjugation Kit

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	1	3	1	-20°C
B	LINK activated Streptavidin	1	3	1	-20°C
C	Reaction Buffer	15 ml	30 ml	30 ml	4-8°C
D	10K MWCO	1	3	1	Room temperature

Overview

MagicLink™ Streptavidin Conjugation Kit is a new generation of streptavidin conjugation kit. The kit enables the instant conjugation of a pre-activated streptavidin to a biopolymer. Using third generation click chemistry, the conjugate forms instantaneously at room temperature. The resulting conjugate is water-soluble and highly stable.



This product can be used to conjugate a variety of compounds with streptavidin. Substrates must contain a primary amine to work. Potential compounds include antibodies, proteins, modified oligonucleotides, or fluorescent polymers with primary amines.

Note: Upon receipt, components A and B must be stored at-20°C. Do not freeze reaction buffer (component C). Warm all components to room temperature before beginning.

- Click chemistry ensures a conjugation efficiency > 90%
- Conjugates are stable and water soluble
- Wide range of target substrates
- No reducing agents or denaturing conditions needed

Simplified Protocol

1. Buffer exchange the target protein or antibody to reaction buffer provided.
2. Dissolve Magic-NHS in a small amount of DMSO.
3. Transfer the required volume of Magic-NHS to antibody solution. Leave reacting at room temperature for >1 hour.
4. Remove excess Magic-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 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.

5. Solvate the Link-Streptavidin with Reaction Buffer.
6. Mix Link-Streptavidin with the activated antibody. Leave reacting >1 hour.
7. (Optional) Analyze results by SDS-PAGE or other method of choice.

Preparation of Working Solution

This kit is optimized to label antibodies at a 100 µg - 1 mg scale per reaction. The ideal antibody concentration should be 1-5 mg/ml. Adjust concentration using the provided reaction buffer.

The antibody (or other target protein) should be pure and free of amines, glycine, BSA, or gelatin. Glycine and other small molecules can be removed by dialysis or by MWCO filtration. Low concentrations of sodium azide (<3 mM) or thimerosal (<1 mM) will not interfere with conjugation. Larger impurities such as gelatin or BSA are not tolerated well.

Antibody Activation Protocol

Dissolve Magic-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 Magic-NHS stock solution to the antibody solution. The amounts are prepared with the

appropriate amount of linker to react with 100 µg or 1 mg of IgG1 antibody depending on the kit chosen. 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 within ~2 hours after purification.

Purification of Activated Antibody using MWCO

1. Dilute the reaction mixture with buffer until the DMSO concentration is below 5% v/v.
2. Transfer 400 µl of DI water into a fresh MWCO filter and centrifuge for 3 minutes at 14,000 x g. Discard the liquid in both the upper and lower chambers.
3. Transfer the reaction mixture from 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-streptavidin 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.

MagicLink Streptavidin-Antibody Conjugation

This instruction applies to 100 µg conjugation kit as well as 1 mg kit as the supplied Link-Streptavidin is to scale.

1. Transfer 100 µl DI water to the Link-Streptavidin (Component B) vial. Gently pipette up and down or vortex to mix. The resulting concentration is 10 mg/ml in the 100 µg kit and 5 mg/ml in the 500 µg kit.
2. Transfer all 100 µl of Link-Streptavidin to the Magic-activated antibody. With 100 µg of antibody and 100 µg Streptavidin provided, this results in a 1:3 antibody/streptavidin ratio. Adjust the volume of Link-Streptavidin to match the needs of downstream applications. Mix by gently pipetting up and down or by vortexing the vial for a few seconds.

   Note: For other target biomolecules, users need optimize the protein/streptavidin mix ratio. This can be done by calculating the number of nmol of a given sample using the molecular weight.

3. Leave the reaction at room temperature for >1 hour. Gentle agitation on a shaker or rotating is ideal.
4. The streptavidin-antibody conjugate is now ready for use.
5. (Optional) Store final conjugate at -20°C or below in a suitable buffer.

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.

Analysis of the Streptavidin Conjugate

The simplest way to confirm conjugation is through SDS-PAGE gel electrophoresis. 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 streptavidin conjugates.

Reaction Details

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

Magic-Antibody Conjugation (example)

Component	MW	Mass (μg)	nmol	Ratio	Volume (μL)	Initial Conc.	Final Conc.
Magic-NHS (in DMSO)	886	100	112.87	16.9	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%

Streptavidin-Antibody Conjugation (example)

Component	MW	Mass (μg)	nmol	Ratio	Volume (μL)	Initial Conc.	Final Conc.
Link-Streptavidin	55,000	1762	32.04	4.8	200	8.8 mg/ml	4.4 mg/ml
Magic-IgG1 Antibody (aq.)	150,000	1000	6.67	1	200	5.0 mg/ml	2.5 mg/ml

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 reaction buffer provided, or 1X PBS 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