

LINK NHS

Component	Product size	Storage
	BP-50064	
LINK NHS	2 μmol	-20 °C

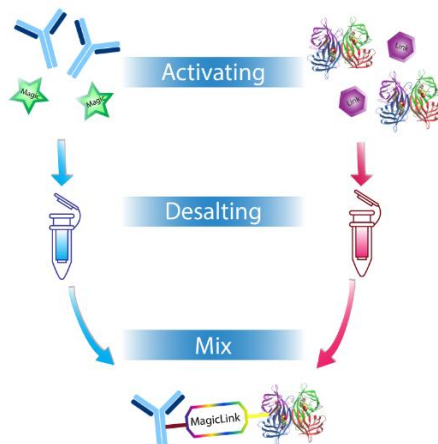
Overview

LINK NHS ester contains NHS ester and LINK functional group with a hydrophilic spacer. This compound can react with amine containing biomolecules such as antibody, protein, enzyme, amine-modified oligonucleotides, etc. at neutral or slightly basic pH to form LINK-activated biomolecules. The spacer helps with hydrophilic and bonding properties of the new biomolecules in bioassay.

Scientist can activate their biomolecules with LINK NHS to get LINK-activated biomolecules which will instantly react with a MAGIC-activated biomolecule to form a new biomolecule by MagicLink™ chemistry. The new linked biomolecule is ready to use.

LINK NHS is one component of BroadPharm proprietary MagicLink™ crosslinking chemistry.

LINK NHS and MAGIC NHS (catalog # BP-50063) are always used together for linking the two biomolecules.



Scheme 1. a typical flow chart of LINK NHS activating protein to form LINK-activated protein (red part)

Technical Considerations

Pre-conjugation considerations for the protein.

- The protein 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.
- Activated protein should be used right away for protein coupling via MagicLink™ chemistry.

Sample Experimental Protocol

1. Reconstitute LINK NHS vial with 100 µl DMSO. Use immediately after reconstitution.
2. Activation
Suggested activation condition:
20X LINK NHS to protein ratio.
Protein concentration between 1-5 mg/ml in 1X PBS pH 7.2 – 7.5.

Sample calculation of protein amount to use for reaction with every 10 µl of LINK NHS in DMSO at 20X LINK to protein ratio.

Protein volume (ml) = protein molecular wt. / protein conc. / 100,000

Unit of molecular weight is g/mol, unit of concentration is mg/ml.

Example calculation for a solution of antibody 150 kD (or 150,000 g/mol) at 5 mg/ml concentration.

ml of antibody to use with 10 µl of LINK NHS (at 20 fold ratio) = $150000 / 5 / 100000 = 0.3$ ml or 300 µl

If a total of 100 µl LINK NHS is to be used up (at 20 fold ratio), then it would require 3 ml of antibody.

Protein activation

Protein should be 1 – 5 mg/ml in 1X PBS (buffer exchange, see note in technical consideration). Add LINK NHS volume according to the calculation above. Mix gently and incubate for 1 hour at room temperature.

Desalt of the Activated Protein

Example of desalting step by Amicon Ultra – 0.5 ml concentrator (not supplied), using a microcentrifuge. Researcher to select appropriate molecular weight cutoff of concentrator, and or different size concentrator; follow manufacturer's direction.

1. Hydrate concentrator membrane 'filter device' with 400 to 500 µl of 1X PBS pH 7.2 – 7.5, or DI water, and microcentrifuge 14,000 x g, for 3 minutes. Discard, liquid from the filter device and collection tube.
2. Spin down by adding activated 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 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 activated protein from the filter device into a microcentrifuge tube.
6. Optional for maximum recovery, add 1X PBS, volume determined by the user, to the filter devices to rinse out residual protein. Microcentrifuge pulse spin, collect proteins/PBS from filter device, and add to the microcentrifuge tube from step 5, mix.

Determine the ratio of the number of LINK group: antibody

Here is a reference for the number of LINK groups after LINK NHS modification reaction:

antibody conc. (mg/ml)	Mixing ratio (MR)	$N_{\text{magic}} / N_{\text{IgG}}$
2	20:1	5~6
10	3:1	1.0~1.5
10	5:1	2.0~2.5
10	10:1	3.0~4.0

The number of LINK groups can be determined with 650-LINK(Cat# BP-50066).

Storage

Activated protein should be used right away for protein coupling via MagicLink™ chemistry. It is not recommended for longtime storage.