MagicLink™ Protein-Protein Crosslinking Kit

Components		Product Size			Storage
		BP-50011	BP-50010	BP-50009	
		1 x 100 µg	3 x100 µg	1 x 1 mg	
A	MAGIC NHS (MW ~900)	1 vial	3 vials	1 vial	-20°C
B	LINK NHS (MW ~900)	1 vial	3 vials	1 vial	-20°C
C	Reaction Buffer	10 ml	30 ml	10 ml	4-8°C
D	DMSO	1 ml	1 ml	1 ml	Room temperature
E	Protein concentrator	N/A	N/A	2	Room temperature
Note: The kit can also be used to crosslink any amine containing biomolecules together, such as, enzyme, oligo, antibodies, etc.					

Overview

MagicLink™ Protein-Protein Crosslinking Kit links two proteins easily and efficiently. The kit is based on BroadPharm's MagicLink™ technology and PEG chemistry. The kit features the most stable, ultra-fast and efficient linkage between two proteins on the market to date. For this cross-linkage reaction, No DTT, TCEP, and reducing agent are needed.

Features:

- High Conjugation efficiency around 95-100%.
- Instant reaction.
- Most stable covalent bonded conjugates on the market.
- A wide range of target proteins – also applicable to antibody fragments and small proteins.
- No DTT, TCEP, and reducing agents needed.
- Stringently QC tested- consistent high quality, excellent consistent from batch to batch

The 1-conjugation kit includes reagents for 1 conjugation of 100 µg of protein. The 3-conjugation kit includes reagents for 3 reaction of 100 µg scale. The 1 mg kit includes 1 conjugation reaction for 1 mg of proteins.

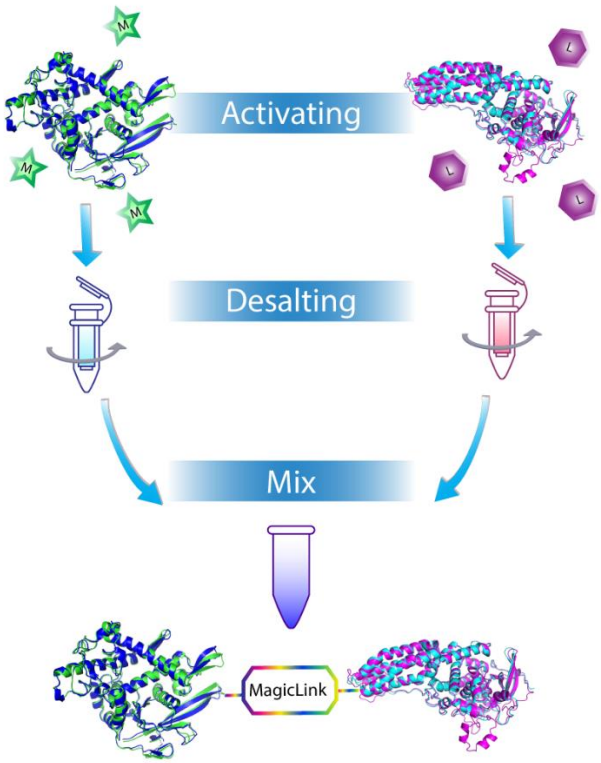

Figure 1 Schematic representation of three step process to prepare protein-protein linkage using MagicLink™ chemistry. Initially protein 1 and 2 is modified to introduce a MAGIC group or LINK group, and subsequently instantly react with each other after removal the excess Magic and Link linkers and mixing.

Technical Considerations

Pre-conjugation considerations for the proteins.

This instruction is for use with kits 100 µg, and 1 mg of protein per reaction. For optimal results, the protein 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 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.

Experimental Protocol (for 100ug and 1mg kit)

1. Reconstitute MAGIC NHS (component A), and LINK NHS (component B) vials, each with 50 µl DMSO.
   Note: the concentrations are 2 nmol/µl for 100 µg kit, and 10 nmol/µl for 1 mg kit.

2. Calculate from Equation 1, the volume of MAGIC NHS to add to the first protein; add and mix.
   Calculate from Equation 1, the volume of LINK NHS to add to the second protein; add and mix.

   Equation 1
   Calculate appropriate volume of activating reagent (MAGIC NHS or LINK NHS) to add to protein solution.

   $$\frac{[(Mass/MW) * 1000] * MR}{Conc}$$ = µl activating reagent to add to sample

   Where
   Mass: antibody mass (µg)
   MW: protein molecular weight (ie 150,000 for antibody).
   MR: activating reagent to antibody ratio; 20 is recommended.
   Conc: the concentration (nmol/µl) of the activating reagent stock solution, (2 nmol/µl for the 100 µg kit, and 10 nmol/µl for the 1 mg kit).

   Note: Different protein may have different labeling efficiency depending on the availability of amine groups.

3. Incubate the two activation reactions at room temperature for 1 hour, preferably with use of a rotator.

Desalt of the Activated Protein 1 and Protein 2 (1 mg kit)

Note:
- 100 µg kit desalting is not necessary, optional if user desires. Concentrator not supplied with the kit. Skip to section 'Generation of Protein-Protein Conjugate' for next step.
- Use two concentrators, one for each protein.

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 activated proteins to the concentrators/filter devices up to 500 µl each. Then, microcentrifuge at 14,000 x g, 8 minutes, or to minimum volume ~ 50 µl left in the filter devices. Discard waste from the collection tubes.
3. Desalt by adding reaction buffer to the filter devices up to 500 µl each. Microcentrifuge at 14,000 x g, 8 minutes, or to minimum volume ~ 50 µl left in the filter devices. Discard waste from the collection tubes.
4. Repeat step 3, twice.
5. Collect activated proteins from filter devices into separate microcentrifuge tubes.
6. Optional, for maximum recovery, add reaction buffer, volume determined by the user, to the filter devices to rinse out residual proteins, microcentrifuge pulse spin, collect proteins/reaction buffers from filter devices, add to their respective microcentrifuge tubes from step 5, mix.
7. Determine sample concentrations, then proceed to the next section.

Generation of Protein-Protein Conjugate

1. Measure the protein 1 and protein 2 concentrations and adjust to same concentration with reaction

buffer.

2. Mix at 1:1 ratio and incubate at room temperature for 1 hour. Conjugations can also be incubated overnight at room temperature with no adverse effect.
3. Your conjugate is now ready for use.

Storage

The long-term stability of the two proteins complex will depend on many factors, including the protein 1 and protein 2 themselves, the storage temperature and conditions. In order to maximize stability, generally, the conjugates can be stored in concentrated solution and at a low temperature. We would suggest checking with the protein manufacturers if their products can be stored in 50% glycerol at - 20°C – you should be able to store most conjugates in these conditions, as long as the they are compatible in their unconjugated forms. If it is appropriate for your reagents and subsequent experiments, the addition of preservatives may also be helpful.

Troubleshooting

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