



Lightning-Link™ HRP Conjugation Kit

701-0010	1 x 100mg LL-HRP	1 labeling reaction, up to 400mg of Ab
701-0000	3 x 100 mg LL-HRP	3 labeling reactions, each up to 400mg of Ab
701-0002	1 x 1mg LL-HRP	1 labeling reaction, up to 4mg of Ab
701-0004	1 x 5mg LL-HRP	1 labeling reaction, up to 20mg of Ab
701-0003	5 x 1mg LL-HRP	5 labeling reactions, each up to 4mg of Ab

Release 008; revised June 2007

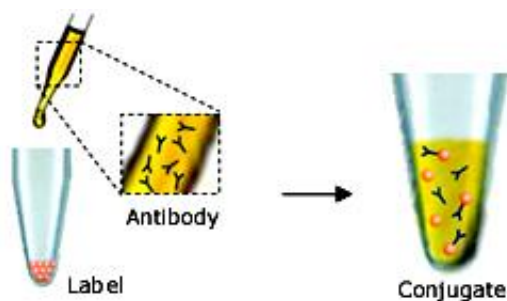
Technical bulletin 1011

1. INTRODUCTION

The Lightning-Link™ HRP kit allows conjugations to set up in seconds, simply by adding a solution of the protein to be labeled to a proprietary lyophilised HRP mixture (Figure 1).

By circumventing the desalting or dialysis steps that commonly interrupt traditional protein conjugation procedures, Lightning-Link™ technology can be used to label small quantities of protein with 100% recovery and no excessive dilution of the conjugate.

Fig. 1 Lightning-Link™ antibody conjugation



Upon dissolution of Lightning-Link™ mixture with a solution of the antibody (or other biomolecule to be labeled) proprietary chemicals in the mixture become activated. This results in the directional coupling of the antibody to the enzyme label in a gentle and controlled process at near-neutral pH. The hands-on time for the entire procedure is typically 20-30 seconds.

Lightning-Link™ makes it possible to label primary antibodies and other proteins with ease, and eliminates the need for secondary reagents in immunoassay procedures such as Western blotting, ELISA and immunocytochemistry.

2. INSTRUCTIONS

2.1 Storage and components

The kit is shipped at ambient temperature in a tamper-evident polypropylene container. Store at -20°C upon receipt.

Kit contents:

Glass vial(s) of Lightning-Link™ mix (1, 3 or 5 vials, depending on pack size)

1 vial of LL-Modifier reagent

1 vial of LL-Quencher reagent

2.2 Considerations before use

2.2.1 Sample buffer

Ideally, the antibody to be labeled should be in 10-50mM amine-free buffer pH range 6.5 to 8.5. However, many buffers outside these limits of concentration and pH can be accommodated. Modest concentrations of Tris buffer are also tolerated. Appendix 1 gives further guidance on buffers and compatible additives.

2.2.2 Amount and volume of antibody

The amount of antibody used for labeling ideally should correspond to molar ratios between 4:1 and 1:1 Ab to HRP. Taking account of the molecular weights (160,000 versus 40,000), this means that for 100µg LL-HRP you need to add between 100-400µg of antibody. The volume of the antibody sample, ideally, should be up to 100µl (100µg pack size), up to 1ml (1mg pack size) and up to 5ml (5mg pack size). Antibody concentrations in the range 0.5-5mg/ml generally give optimal results, but concentrations and

volumes outside these suggested ranges have also yielded excellent conjugates.

2.3 Setting up conjugation reactions

2.3.1. Before you add antibody to the Lightning-Link™ mix, add 1µl of LL-Modifier reagent for each 10µl of antibody to be labeled. Mix gently.

2.3.2. Remove the screw cap from the vial of Lightning-Link™ mix and pipette the antibody sample (with added LL-modifier) directly onto the lyophilised material. Resuspend *gently* by withdrawing and re-dispensing the liquid once or twice using a pipette.

2.3.3. Place the cap back on the vial and leave the vial standing for 3 hours at room temperature (20-25°C). Alternatively, and sometimes more conveniently, conjugations can be set up and left overnight, as the longer incubation time has no negative effect on the conjugate.

2.3.4. After incubating for 3 hours (or more), add 1µl of LL-quencher reagent for every 10µl of antibody used. The conjugate can be used after 30 minutes.

2.4 Storage of conjugates

For any new conjugate, initial storage at 4°C is recommended. A preservative may be desirable for long-term storage. Other storage conditions (e.g. frozen at -70°C or stored at -20°C with 50% glycerol) may also be satisfactory. The best conditions for any particular conjugate must be determined by experimentation.

Appendix 1. Compatibility of buffers and buffer additives.

Amine-free buffers, including MES, MOPS, HEPES and phosphate are compatible if they are in the concentration range 10-50mM and have pH values in the range 6.5-8.5, as the addition of LL-Modifier provides the conditions necessary for efficient conjugation. Common non-buffering salts (e.g. sodium chloride), chelating agents (e.g. EDTA), and sugars may be present, as they have no effect on conjugation efficiency. Azide (0.02-0.1%) has little or no effect.

If the amine-free buffer is relatively concentrated and outside the pH range 6.6-8.5 you may need to add more LL-modifier for each 10µl of antibody. Excess LL-Modifier is provided so that you can check the pH of the buffer after the

addition of the modifier. Ideally the pH should be around 7.3-7.6, though efficient conjugation occurs anywhere between pH 6.8 and 7.8. Avoid buffer components that are nucleophilic, as these may react with Lightning-Link™ chemicals. Primary amines (e.g. amino acids, ethanolamine) and thiols (e.g. mercaptoethanol, DTT) fall within this class. (Note: Unusually, for an amine, Tris has little effect on conjugation efficiency as long as the concentration is 20mM or less).

Q1. What functional groups do I need on my protein?

Lightning-Link™ requires amine groups on the molecule to be labeled.

Q2. How much antibody should I use?

There is no 'right' answer here and it is possible to generate excellent conjugates using a range of conditions. If there is a specific reason for wanting a conjugate of relatively low molecular weight (with 1 or 2 HRP's attached), add 300-400µg of antibody (to 100µg of LL-HRP). To introduce more HRP to increase assay sensitivity use 100-200µg of antibody. Free LL-HRP is completely benign in applications that involve wash steps (e.g. ELISA westerns) but the concentration of free label can be minimized, if required, by using >200µg of antibody.

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