

Innova Biosciences

Lightning-Link™ Alkaline Phosphatase Conjugation Kit

702-0030	3 x 10 µg	LL-AP conjugation kit
702-0005	1 x 100 µg	LL-AP conjugation kit
702-0010	3 x 100 µg	LL-AP conjugation kit
702-0015	1 x 1mg	LL-AP conjugation kit

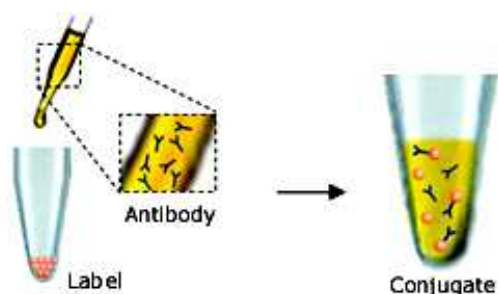
Release 005; February 2012

1. INTRODUCTION

The Lightning-Link™ conjugation kit allows AP conjugations to set up in seconds, simply by adding a solution of the protein to be labeled to a proprietary lyophilised mixture containing AP (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 without 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 or 3 vials, depending on pack size)

1 vial of LL-AP Modifier reagent

1 vial of LL-AP Quencher reagent

2.2 Considerations before use

2.2.1 Sample buffer

The purified antibody to be labeled should ideally be in 10-50mM amine-free buffer (e.g. MES, MOPS, HEPES, PBS), pH range 6.5 to 8.5. If the buffer is more concentrated or outside this pH range contact the technical support team.

Common non-buffering salts (e.g. sodium chloride), chelating agents (e.g. EDTA), and sugars have no effect on conjugation efficiency. Azide (0.02 to 0.1%) and BSA (0.1 to 0.5%) have little or no effect. Glycerol up to 50% has no effect.

Avoid buffer components that are nucleophilic, as these may react with Lightning-Link™ chemicals. Primary amines (e.g. amino acids or ethanolamine) and thiols (e.g. mercaptoethanol or DTT) fall within this class. (Note: Tris-based buffers should be avoided.)

2.2.2 Amount and volume of antibody

The amount of antibody used for labeling should be the same as the pack size of LL-AP. e.g. For 100µg LL-AP add 100µg of antibody. The volume in which the antibody is added ideally should be 4µl-10µl (10µg pack size), 40µl-100µl (100µg pack size), and 0.40-1ml (1mg pack size). Antibody concentrations in the range 1-4mg/ml are therefore ideal. For any new antibody, optimization of the ratio of antibody to AP is often worthwhile.

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.

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

Lightning-Link™ requires amine groups on the molecule to be labeled. Most proteins have lysine and/or alpha-amino groups. All antibodies will have multiple amine functions.

Q2. Do I need to purify the conjugate?

No. The chemicals used in Lightning-Link™ are deactivated by the quencher, and the by-products are benign and do not need to be removed.

Technical support team

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