

## Lightning-Link™ AMCA Conjugation Kit

712-0010 3 x 100 mg LL-AMCA conjugation kit  
712-0015 1 x 1 mg LL-AMCA conjugation kit

Release 002 September 2008

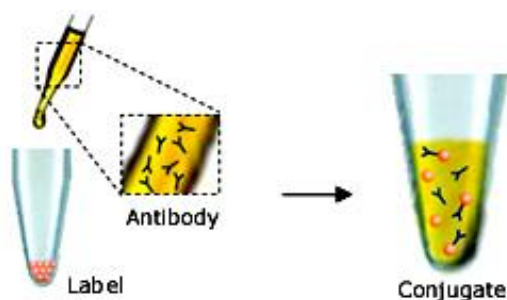
Technical bulletin 1293

### 1. INTRODUCTION

The Lightning-Link™ conjugation kit allows AMCA conjugations to set up in seconds, simply by adding a solution of the protein to be labeled to a proprietary lyophilised mixture containing AMCA (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 fluorescent 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- Modifier reagent

1 vial of LL- Quencher FD 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 should be the same as the pack size of LL-AMCA. e.g. For 100µg LL-AMCA add 100µg of antibody. The volume in which the antibody is added ideally should be 25µl-100µl (100µg pack size), and 0.25-1ml (1mg pack size). Antibody concentrations in the range 1-4mg/ml are therefore ideal. However, concentrations and volumes outside these suggested limits have also yielded excellent conjugates. For any new antibody, optimization of the ratio of antibody to AMCA 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 FD 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 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 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: 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. Most proteins have lysine and/or alpha-amino groups. All antibodies will have multiple amine functions.

## Q2. Can non-antibody molecules be labeled?

Yes. While labeling of antibodies is a major application, the only requirement is that the protein to be labeled has amine functionality.

## Q3. 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.

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