



## Lightning-Link™ Cy3.5 Conjugation Kit

784-0010 3 x Ab labelings (each 100-200mg scale)

784-0015 1 x Ab labeling (1-2mg scale)

Release 002; revised October 2008

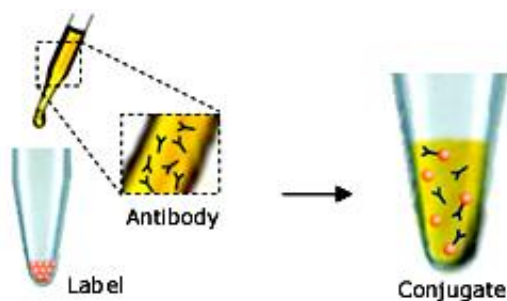
Technical bulletin 7844

### 1. INTRODUCTION

The Lightning-Link™ conjugation kit allows fluorescent conjugations to be set up in *seconds*, simply by adding a solution of the protein to be labeled to the lyophilised mixture containing a proprietary activated fluorescent ligand (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 coupling of the antibody to the fluorescent dye, in a gentle and controlled process at near-neutral pH. Lightning-Link™ makes it possible to conjugate primary antibodies and other proteins with ease, using a simple, efficient process that has safeguards to prevent over-labeling of the biomolecule and that ensures 100% recovery even at small scale.

### 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:

1 or 3 glass vial(s) of Lightning-Link™ mix

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 recommended amount of antibody to be used for labeling is 100-200µg for 784-0010 and 1-2mg for 784-0015. The volume of the antibody sample, ideally, should be in the range 40-100µl (784-0010), and 400-1000µl (784-0015). Antibody concentrations of 1-4 mg/ml generally give optimal results, but concentrations and volumes outside the 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 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 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: Tris has little effect on conjugation efficiency as long as the concentration is 20mM or less).

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