



Lightning-Link™ R-Phycoerythrin Conjugation Kit

703-0005	1 x 100 mg	LL-RPE conjugation kit
703-0010	3 x 100 mg	LL-RPE conjugation kit
703-0015	1 x 1mg	LL-RPE conjugation kit

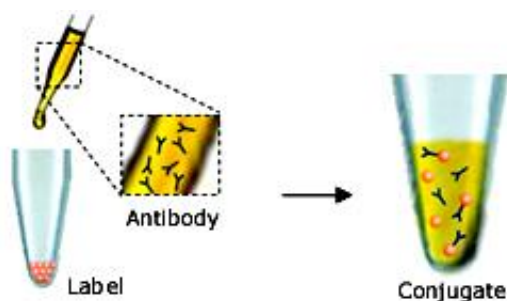
Release 004; September 2008

Technical bulletin 7058

1. INTRODUCTION

The Lightning-Link™ conjugation kit allows PE conjugations to set up in seconds, simply by adding a solution of the antibody to be labeled to a proprietary lyophilised mixture containing PE (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 for FACS analysis with 100% recovery.

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, covalent* bonding 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 usually 20-30 seconds.

Lightning-Link™ makes it possible to label antibodies with PE with ease, and eliminates the need for secondary reagents in FACS experiments. Direct labeling can simplify and improve data quality in multicolor experiments by eliminating problems caused by dissociation and crossover of secondary reagents.

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-PE Modifier reagent

1 vial of LL-PE 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

In view of the large size of PE (240kDa), the amount of antibody used in a labeling reaction must always be less than the pack size of LL-PE, in order that the PE is in a slight molar excess. The best ratio for any new antibody reagent must be determined by experimentation but 50-60µg of IgG antibody for every 100µg of LL-PE usually gives optimal results. The 60µg quantity corresponds to an Ab:PE molar ratio of 1:1.

The volume in which the antibody is added ideally should be around 40µl (100µg pack size), and around 400µl (1mg pack size). Where the

concentration of antibody is relatively low, and where it is impractical to concentrate the antibody, up to twice the volume stated above (i.e. 80µl for the 100µg PE pack size) may be added without any significant loss in conjugation efficiency.

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 it standing for 3 hours in the dark at room temperature (20-25°C). Alternatively, and often 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, storage at 4°C is recommended. A preservative may be desirable for long-term storage. Other storage conditions 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 have no effect on conjugation efficiency. Azide (0.02-0.1%) has little or no effect. Glycerol up to 50% has 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. 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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