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704-0030 Protocol

Lightning-Link Type A Biotin Antibody Labeling Kit [Biotin] (704-0030)

1. INTRODUCTION

Lightning-Link(TM) Biotin Conjugation Kit (Type A*)
*Optimized for assays in which a streptavidin-labeled reagent will be used

The Lightning-Link conjugation kit allows biotinylations to be set up in seconds, simply by adding a solution of the protein to be labeled to a lyophilised mixture containing a proprietary activated biotin ligand. 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.

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 biotin, which has an extended spacer, in a gentle and controlled process at near-neutral pH. Lightning-Link makes it possible to biotinylate 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 degrees Celsius upon receipt.

Kit contents:
1 or 3 glass vial(s) of Lightning-Link(TM) mix 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 recommended amount of antibody to be used for labeling is 100-200ug for 704-0010 and 1-2mg for 704-0015. The volume of the antibody sample, ideally, should be in the range 40-100ul (704-0010), and 400-1000ul (704-0015). 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 1ul of LL-Modifier reagent for each 10ul 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 degrees Celsius). 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 1ul of LL-quencher reagent for every 10ul of antibody used. The conjugate can be used after 30 minutes.

2.4 Storage of conjugates

For any new conjugate, initial storage at 4 degrees Celsius is recommended. A preservative may be desirable for long-term storage. Other storage conditions (e.g. frozen at -70 degrees Celsius or stored at -20 degrees Celsius 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 10ul 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. Moreover, the conjugation efficiency is very high and, unlike other biotin reagents, LL-biotin does not hydrolyze in solution or during storage, thus it is not necessary to use a large excess or to purify the final conjugate.

Q3. 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.