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780-0010 Protocol

Lightning Link Cy3 Conjugation Kit Protocol (780-0010)

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. 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 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

- Considerations before use

- 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.

- Amount and volume of antibody

The recommended amount of antibody to be used for labeling is 10-20ug for 780-0030, 100-200ug for 780-0010 and 1-2mg for 780-0015. The volume of the antibody sample, ideally, should be in the range 4-10ul (780-0030), 40-100ul (780-0010), and 400-1000ul (780-0015). Antibody concentrations of 1-4 mg/ml generally give optimal results, but concentrations and volumes outside the suggested ranges have also yielded good conjugates.

- Setting up conjugation reactions

- 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.

- 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.

- 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.

- After incubating for 3 hours (or more), add 1ul of LL-quencher FD reagent for every 10ul of antibody used. The conjugate can be used after 30 minutes.

- 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).