Product Datasheet

Lightning-Link (R) FluoProbes647H Antibody Labeling Kit 791-0010

Unit Size: 3 x .200mg Reactions

Store at -20C. Avoid freeze-thaw cycles.

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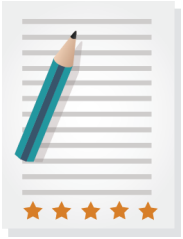

791-0010

Lightning-Link (R) FluoProbes647H Antibody Labeling Kit

Product Information

Unit Size	3 x .200mg Reactions
Concentration	Concentration is not relevant for this product. Please see the protocols for proper use of this product.
Storage	Store at -20C. Avoid freeze-thaw cycles.
Conjugate	FluoProbes 647H

Product Description

Description	Lightning-Link is an innovative technology that enables direct labeling of proteins, peptides or other biomolecules. **Key Features:** • Easy to use • Requires 30 sec hands-on time • No spin or separation steps involved The researcher simply pipettes the antibody or other biomolecule into a vial of lyophilized mixture containing the label of interest and incubates for either 3 hours or See Lighning-Link Rapid for only 15 min incubation. Despite its apparent simplicity, the Lightning-Link process is sophisticated and generates conjugates with performance characteristics identical to, or better than, those prepared with laborious multistep conjugation procedures.
Kit Components	1 or 3 glass vial(s) of Lightning-Link mix, 1 vial of LL-Modifier reagent, 1 vial of LL-Quencher reagent
Notes	Learn more about Lightning-Link™ Conjugation Kits by reading FAQs For more information please check out these useful links! Fluorescent Labels Poster Antibody Labeling Guide Antibody Purification Guide Lightning Link® is a registered trademark of Innova Biosciences. This product is manufactured by Expedeon Inc. and distributed by Novus Biologicals.

Product Application Details

Application Notes	By circumventing the desalting or dialysis steps that commonly interrupt traditional antibody conjugation procedures, LightningLink technology can be used to label both small (e.g. 10 ug) and large quantities of primary antibodies with ease. Batch-to-batch variation upon scale up is minimal as the process is so simple, and recoveries are always 100%. This kit is supplied with 3 vials, each suitable for labeling up to 200 ug of antibody.

Procedures

Antibody Labeling Guide (791-0010)
Our Lightning-Link Antibody Labeling Guide is a user friendly tool that allows you to learn the basics of common antibody labeling methods. It also describes Lightning-Link technology, which massively simplifies the production of labeled antibodies. The Lightning-Link approach requires no knowledge of chemistry and the hands-on time is just 30 seconds.

Visit this link to read our Antibody Labeling Guide:
http:///support/support-by-application/antibody-conjugation/illustrated-assay.html

This How To Guide Covers:
- Types of Immuno-experiments and Associated Labels
- Direct vs. Indirect Detection Methods
- Antibody Labeling Methods
- Buffers and Additives
- Antibody Concentration and Purity
- Benefits of Lightning-Link Antibody Labeling Kit

Lightning-Link FluoProbes647H Antibody Labeling Kit Protocol (791-0010)
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 100-200ug for 791-0010 and 1-2mg for 791-0015. The volume of the antibody sample, ideally, should be in the range 40-100ul (791-0010), and 400-1000ul (791-0015). Antibody concentrations of 1-4 mg/ml generally give optimal results, but concentrations and volumes outside the suggested ranges have also yielded excellent conjugates.

- Setting up conjugation reactions

- Before you add antibody to the Lightning-Link mix, add 1ul of LL-Modifier reagent for each 10ml 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 (20-25C). 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 10ml of antibody used. The conjugate can be used after 30 minutes.

- Storage of conjugates

For any new conjugate, initial storage at 4C is recommended. A preservative may be desirable for long-term storage. Other storage conditions (e.g. frozen at -70C or stored at -20C 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).

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