Product Datasheet

Lightning-Link R-PE Antibody Labeling Kit
703-0030

Unit Size: 3 x 10ug Reaction

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

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Publications: 39

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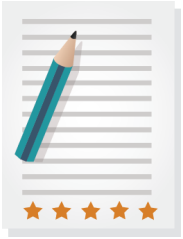
Updated 10/17/2016 v.20.1



703-0030

Lightning-Link R-PE Antibody Labeling Kit

Product Information

Unit Size	3 x 10ug Reaction
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	PE

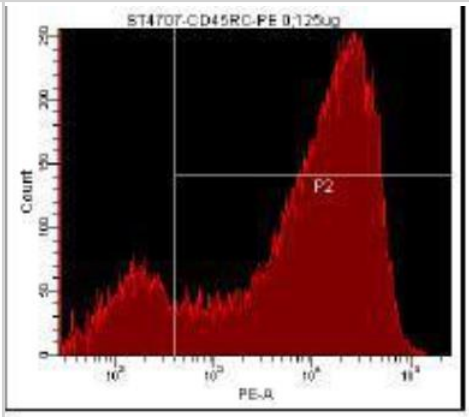
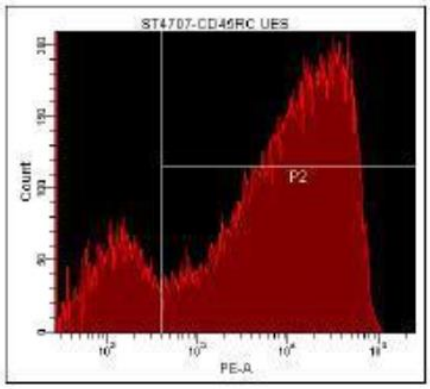
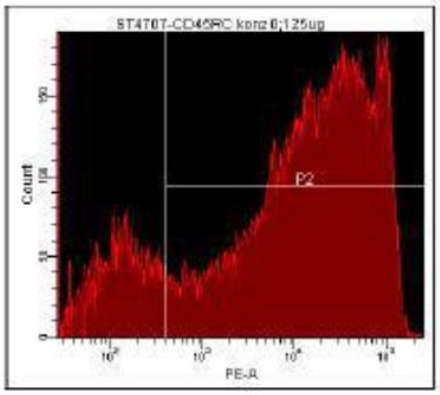
Product Description

Description	Lightning-Link® is an innovative technology that enables direct labeling of antibodies, proteins, peptides or other biomolecules for use in R&D applications. **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. R-Phycoerythrin (R-PE) is a fluorescent protein from the phycobiliprotein family, and is isolated from red algae. The absorbance spectrum of R-PE covers a broad range of excitation wavelengths, which provides an advantage for multi-color immunofluorescent staining or cell sorting. R-PE is one of the most intensely fluorescent phycobiliprotein having orange fluorescence. It is significantly brighter and more photostable than conventional organic fluorophores and it has a high quantum yield.
Kit Components	Glass vial(s) of Lightning-Link mix (1 or 3 vials, depending on pack size), 1 vial of LL-AP Modifier reagent, 1 vial of LL-AP 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.

Product Application Details

Applications	Flow Cytometry
Recommended Dilutions	Flow Cytometry
Application Notes	This kit contains 3x10ug vials.

Images

Flow Cytometry: Lightning-Link R-PE Antibody Labeling Kit [703-0030] - Direct labeling with Lightning Link CD45RC-PE	
Flow Cytometry: Lightning-Link R-PE Antibody Labeling Kit [703-0030] - Indirect labeling with CD45RC supernatant	
Flow Cytometry: Lightning-Link R-PE Antibody Labeling Kit [703-0030] - Indirect labeling with purified CD45RC antibody	

Publications

Nagaraj V, King B, Storm P et al. Complement inhibitor CD55 governs the integrity of membrane rafts in pancreatic beta cells, but plays no role in insulin secretion Biochem Biophys Res Commun. 2015 May 8 [PMID: 25797618] (FLOW)

Karagiannis P, Villanova F, Josephs DH et al. Elevated IgG4 in patient circulation is associated with the risk of disease progression in melanoma Oncoimmunology 2015 Jun 3 [PMID: 26451312] (FLOW)

Yu X, Rui L, Shao Q et al. Changes of CD4+CD25+ Cells Ratio in Immune Organs from Chickens Challenged with Infectious Bursal Disease Virus Strains with Varying Virulences Viruses 2015 Mar 20 [PMID: 25803101] (FLOW)

Barnable P, Calenda G, Bonnaire T et al. MIV-150/Zinc Acetate Gel Inhibits Cell-Associated Simian-Human Immunodeficiency Virus Reverse Transcriptase Infection in a Macaque Vaginal Explant Model Antimicrob Agents Chemother. 2015 Jul [PMID: 25870063] (FLOW)

Pieper IL, Radley G, Chan CH et al. Quantification methods for human and large animal leukocytes using DNA dyes by flow cytometry Cytometry A. 2016 Jun [PMID: 27271958] (FLOW)

Bigler MB, Egli SB, Hysek CM et al. Stress-Induced In Vivo Recruitment of Human Cytotoxic Natural Killer Cells Favors Subsets with Distinct Receptor Profiles and Associates with Increased Epinephrine Levels: e0145635 PLoS One 2015 Dec 23 [PMID: 26700184] (FLOW)

Starkie DO, Compson JE, Rapecki S, Lightwood DJ. Generation of Recombinant Monoclonal Antibodies from Immunised Mice and Rabbits via Flow Cytometry and Sorting of Antigen-Specific IgG+ Memory B Cells PLoS One 2016 Mar 29 [PMID: 27022949] (FLOW)

Ding J, Tasker C, Lespinasse P et al. Integrin [alpha]4[beta]7 expression increases HIV susceptibility in activated cervical CD4+ T cells via an HIV attachment-independent mechanism. J Acquir Immune Defic Syndr. 2015 Aug 15 [PMID: 26167616] (FLOW)

Rodgers JM, Robinson AP, Rosler ES et al. IL-17A activates ERK1/2 and enhances differentiation of oligodendrocyte progenitor cells. Glia 2014 [PMID: 25557204]

Robinson AP, Rodgers JM, Goings GE, Miller SD. Characterization of Oligodendroglial Populations in Mouse Demyelinating Disease Using Flow Cytometry: Clues for MS Pathogenesis. PLoS One 2014 [PMID: 25247590] (FLOW)

Bates JM, Flanagan K, Mo L et al. Dendritic cell CD83 homotypic interactions regulate inflammation and promote mucosal homeostasis. Mucosal Immunol 2014 [PMID: 25204675] (FLOW)

Saresella M, Piancone F, Marventano I et al. A role for the TIM-3/GAL-9/BAT3 pathway in determining the clinical phenotype of multiple sclerosis. FASEB J. 2014 [PMID: 25091272]

More publications at http://www.novusbio.com/703-0030

Procedures

Lightning Link R-Phycoerythrin Conjugation Kit Protocol (703-0030)
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. By circumventing the desalting or dialysis steps that commonly interrupt traditional protein conjugation rocedures, Lightning-
Link technology can be used to label small quantities of protein for FACS analysis with 100% recovery.

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 degrees celsius upon receipt.

Kit contents:
Glass vial(s) of Lightning-Link(TM) mix (1 or 3 vials, depending on pack size)
1 vial of LL-PE Modifier reagent
1 vial of LL-PE Quencher reagent

- 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

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 50-60ug of IgG antibody for every 100ug of LL-PE usually gives optimal results. The 60ug quantity corresponds to an Ab:PE molar ratio of 1:1. The volume in which the antibody is added ideally should be around 40ul (100ug pack size), and around 400ul (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. 80ul for the 100ug PE pack size) may be added without any significant loss in conjugation efficiency.

- 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 it standing for 3 hours in the dark at room temperature (20-25 degrees Celsius). 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.

- 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.

- Storage of conjugates

For any new conjugate, storage at 4 degrees Celsius 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 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).

Antibody Labeling Guide (703-0030)
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://www.novusbio.com/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

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