Product Datasheet

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

Unit Size: 3 x 10ug Reaction

Store at -20C.

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Reviews: 1 Publications: 47

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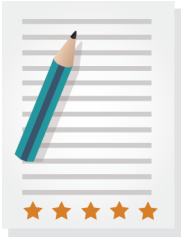
www.novusbio.com/703-0030

Updated 8/21/2018 v.20.1



703-0030

Lightning-Link (R) 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.
Conjugate	PE

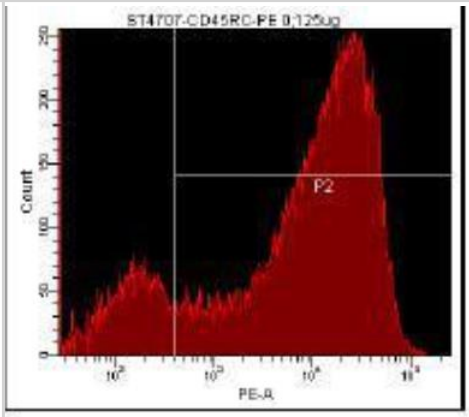
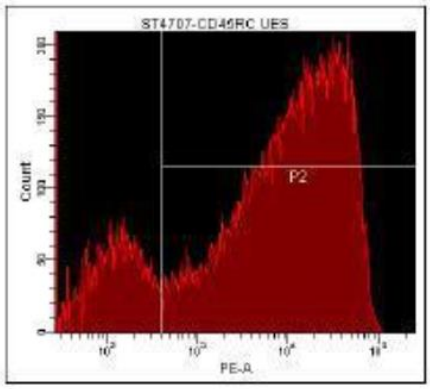
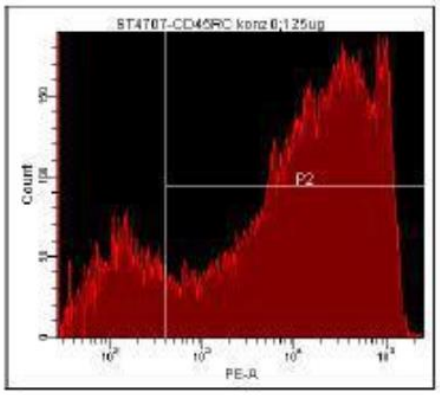
Product Description

Description	Lightning-Link antibody labeling kits enable the direct labeling of antibodies, proteins, peptides or other biomolecules for use in R&D applications, drug discovery and the development of diagnostic kits (See protocol for further information). Our R-PE antibody labeling kit enables the direct conjugation of R-PE to any biomolecule with an available amine group. The researcher simply pipettes the antibody or other biomolecule into the vial of Lightning-Link R-PE and incubates for 3 hours. **FeaturesBenefits**Quick and easy to useSave time, no special knowledge requiredNo separation steps100% recovery - no antibody/protein lossCan be used in a wide range of applicationsFlexibleFreeze driedShips at ambient temperature, long shelf-lifeFully scalable (10 ug to 1 g or more)Easy transfer from R&D to manufacturingStringently QC testedConsistent high quality, excellent batch-to-batch reproducibilityLarge number of labels available Experimental flexibilityReliable: nearly 300 referencesSuccessfully used in many fields of research R-Phycoerythrin (R-PE) is a fluorescent protein from the phycobiliprotein family, present in red algae and cryptophytes. It has three maximal absorbance values of 498, 544 and 566nm (the optimal will depend on the application), and it has a strong emission peak at 580nm. RPE is closely related to **B-Phycoerythrin (B-PE)** and these are the most intense fluorescent phycobiliproteins providing an orange fluorescence.
Kit Components	1 or 3 or 5 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! Antibody Labeling Guide Antibody Conjugation Illustrated Assay This product is manufactured by Expedeon Inc. and distributed by Novus Biologicals.

Product Application Details

Applications	Flow Cytometry
Recommended Dilutions	Flow Cytometry
Application Notes	The recommended conjugation conditions are based on using a 1mg/ml antibody concentration and are designed to give a 1:1 Ab:RPE conjugation molar ratio. Antibodies greater than 1mg/ml should be diluted to 1mg/ml using either milli-Q water or PBS. This kit is supplied with 3 vials, each suitable for labeling up to 10 ug of antibody.

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

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Jelsma T, van der Wal FJ, Fijten H et al. Pre-screening of crude peptides in a serological bead-based suspension array. J Virol Methods. 2017 [PMID: 28545817]

Charlermroj R, Makornwattana M, Himananto O et al. An accurate, specific, sensitive, high-throughput method based on a microsphere immunoassay for multiplex detection of three viruses and bacterial fruit blotch bacterium in cucurbits. J Virol Methods. 2017 [PMID: 28502647]

Tafalla C, Gonzalez L, Castro R, Granja AG. B Cell-Activating Factor Regulates Different Aspects of B Cell Functionality and Is Produced by a Subset of Splenic B Cells in Teleost Fish. Front Immunol. 2017 Mar 15 [PMID: 28360916]

Hadadi E, Zhang B, Baidzajevas K et al. Differential IL-1beta secretion by monocyte subsets is regulated by Hsp27 through modulating mRNA stability. Sci Rep. 2016 Dec 15 [PMID: 27976724]

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Gao Y, Pallister J, Lapierre F et al. A rapid assay for Hendra virus IgG antibody detection and its titre estimation using magnetic nanoparticles and phycoerythrin. J Virol Methods. 2015 Sep 15 [PMID: 26141730]

Frederick JR, Fitzpatrick JR 3rd, McCormick RC et al. Stromal Cell-Derived Factor-1alpha Activation of Tissue-Engineered Endothelial Progenitor Cell Matrix Enhances Ventricular Function After Myocardial Infarction by Inducing Neovasculogenesis. Circulation. 2010 Sep 14 [PMID: 20837901]

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)

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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