

Innova Biosciences

Lightning-Link™ PerCP-Cy5.5 Tandem Conjugation Kit

763-0005 1 x 100 mg LL-PerCP-Cy5.5 Tandem conjugation kit

763-0010 3 x 100 mg LL-PerCP-Cy5.5 Tandem conjugation kit

763-0015 1 x 1mg LL-PerCP-Cy5.5 Tandem conjugation kit

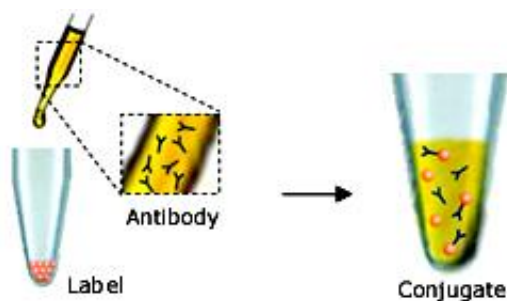
Release 001; November 2008

Technical bulletin 6319

1. INTRODUCTION

The Lightning-Link™ conjugation kit allows PerCP-Cy5.5 tandem conjugations to set up in seconds, simply by adding a solution of the antibody to be labeled to a proprietary lyophilised mixture containing PerCP-Cy5.5 (Figure 1). 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 for FACS analysis with 100% recovery.

Fig. 1 Lightning-Link™ antibody conjugation



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 PerCP-Cy5.5 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°C upon receipt.

Kit contents:

Glass vial(s) of Lightning-Link™ mix (1 or 3 vials, depending on pack size)

1 vial of LL-Modifier reagent

1 vial of LL-Quencher FD 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.

2.2.2 Amount and volume of antibody

The amount of antibody used for labeling should be the same as the pack size of LL-PerCP-Cy5.5 e.g. For 100µg LL-PerCP-Cy5.5 add 100µg of antibody. The volume in which the antibody is added ideally should be 25µl-100µl (100µg pack size), and 0.25-1ml (1mg pack size). Antibody concentrations in the range 1-4mg/ml are

therefore ideal. However, concentrations and volumes outside these suggested limits have also yielded excellent conjugates. For any new antibody, optimization of the ratio of antibody to PerCP-Cy5.5 is often worthwhile

2.3 Setting up conjugation reactions

2.3.1. Before you add antibody to the Lightning-Link™ mix, add 1µl of LL-Modifier reagent for each 10µl 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 it standing for 3 hours in the dark at room temperature (20-25°C). 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.

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

2.4 Storage of conjugates

For any new conjugate, storage at 4°C 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 10µl 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).

Limited use license

Innova Biosciences' Lightning-Link™ conjugation kits are offered for research purposes alone, and are not intended for human, therapeutic or diagnostic use. The purchase of this conjugation kit conveys to the buyer (whether the buyer is a not-for-profit, academic or for-profit entity) the non-transferable right to use the amount of product purchased and the components of the product for in-house research. The buyer shall not sell or otherwise transfer this product, its components, or materials prepared therefrom to any third party. The buyer shall not use this product or its components for commercial purposes. For the avoidance of doubt, 'commercial purposes' means any activity by a party for consideration and includes, without limitation, use of the product or its components (i) in the manufacturing of conjugated materials (e.g. labeled antibodies), (ii) to provide a service, information or data, (iii) for therapeutic, diagnostic or prophylactic purposes, or (iv) for repackaging/resale, whether or not such product or its components are resold for use in research. The use of this product by the buyer constitutes agreement with the terms of this limited use label license for Lightning-Link™ products.

For information on purchasing a license for commercial applications contact Innova Biosciences Ltd, Business Development Office, Babraham Hall, Babraham, Cambridge, UK, CB22 3AT. Tel +44(0)1223 496170; Fax +44(0)1223 496172.

This material is also subject to proprietary rights of GE Healthcare Bio-Sciences Corp. and Carnegie Mellon University and made and sold under License from GE Healthcare Bio-Sciences Corp. This product is licensed for sale only for research. It is **NOT** licensed for any other use. There is no implied license hereunder for any commercial use

COMMERCIAL USE shall include:

- 1 Sale, lease, license or other transfer of the material or any material derived or produced from it.
- 2 Sale, lease, license or other grant of rights to use this material or any material derived or produced from it.
- 3 Use of this material to perform services for a fee for third parties.

If you require a commercial license to use this material and do not have one return this material unopened to Innova Biosciences, Babraham Hall Babraham, Cambridge CB22 3AT, UK and any money paid for the material will be refunded.