



Lightning-Link® HRP Conjugation Kit

Applicable to:

701-0030 3 vials (to label 10-40µg Ab per vial)
701-0010 1 vial (to label 100-400µg Ab per vial)
701-0000 3 vials (to label 100-400µg Ab per vial)

701-0002 1 vial (to label 1-4mg Ab per vial)
701-0003 5 vials (to label 1-4mg Ab per vial)
701-0004 1 vial (to label 5-20mg Ab per vial)

Release 18

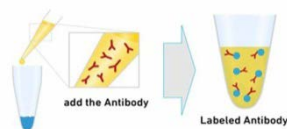
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Introduction

Lightning-Link® technology works by targeting amine groups and can therefore be used for most biomolecules including antibodies, proteins and peptides. Please see the FAQs section of this protocol for recommended adjustments for biomolecules that differ in size from IgGs, including proteins and peptides.

The Lightning-Link® Horseradish Peroxidase (HRP) conjugation kit allows HRP conjugations to be set up in seconds, simply by adding a solution of the antibody (or other biomolecule to be labeled) to a lyophilised mixture containing a proprietary activated HRP ligand (Figure 1).

Figure 1. Lightning-Link® antibody conjugation



By circumventing the desalting or dialysis steps that commonly interrupt traditional protein conjugation procedures, Lightning-Link® technology can be used to label small and large quantities of protein (10µg to 20mg) with 100% recovery.

Upon dissolution of the 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 coupling of the antibody to the HRP label in a gentle and controlled process at near-neutral pH. The hands-on time for the entire procedure is typically 20-30 seconds.

Lightning-Link® makes it possible to label primary antibodies and other proteins with ease, and eliminates the need for secondary reagents in immunoassay procedures such as western blotting, ELISA and immunocytochemistry.

Kit contents

- 1, 3 or 5 glass vial(s) of Lightning-Link® mix
- 1 vial of LL-modifier reagent
- 1 vial of LL-quencher reagent

Instructions

Setting up your conjugation reaction

1. Before you add antibody to the Lightning-Link® mix, add 1µl of LL-modifier reagent for each 10µl of antibody to be labelled. Mix gently.
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.
3. Place the cap back on the vial and leave the vial standing for 3 hours* at room temperature (20-25°C). Alternatively, and sometimes more conveniently, conjugations can be set up and left at 4°C overnight, as the longer incubation time has no negative effect on the conjugate.
***To label 5-20mg of antibody with kit 701-0004, it is important to incubate overnight.**
4. After incubating for 3 hours (or more), add 1µl of LL-quencher reagent for every 10µl of antibody used. The conjugate can be used after 30 minutes. No separation steps are necessary.

Amount and volume of antibody

The amount of antibody used for labeling ideally should correspond to molar ratios between 1:1 and 1:4 Ab to HRP.

Product code	Amount of antibody per vial	Recommended antibody volume per vial
701-0030	10-40µg	4µl-10µl
701-0000 701-0010	100-400µg	40µl-100µl
701-0002 701-0003	1-4mg	0.40-1ml
701-0004	5-20mg	1-5ml

Antibody concentration should be between 0.5mg/ml and 5mg/ml.

Shipping conditions

The kit is shipped at ambient temperature in a tamper-evident polypropylene container.

Store the kits at -20°C upon receipt. Please note that the modifier and quencher can be stored at either 4°C or -20°C.

Buffer considerations

Please see the below table for recommended buffer conditions and components:

Buffer components	
pH	6.5-8.5
Amine free buffer (e.g. MES, MOPS, HEPES, PBS)	✓
Non-buffering salts (e.g. sodium chloride)	✓
Chelating agents (e.g. EDTA)	✓
Sugars	✓
Glycerol	<50%
Thiomersal / Thimerosal	✗
Merthiolate	✗
Sodium Azide	✗
BSA ^{1,2}	<0.1%
Gelatin ^{1,2}	<0.1%
Tris	<50mM
Glycine	✗
Proclin	✗
Borate buffer	✓
Nucleophilic components (Primary amines e.g. amino acids or ethanolamine and thiols e.g. mercaptoethanol or DTT)	✗

¹ Please note that individually the concentrations shown should not affect the reaction. However in combination with additional compounds that are not recommended above a certain concentration, the reaction may be affected.

² If intending to use this kit for immunohistochemistry, it is recommended that there be no gelatin or BSA present.

Storage of conjugates

For any new conjugate, initial storage at 4°C is recommended for 12-18 months*. A preservative may be desirable for long-term storage up to 2 years. Other storage conditions (e.g. frozen at -70°C or stored at -20°C with 50% glycerol) may also be satisfactory. The best conditions for any particular conjugate must be determined by experimentation.

*As long as the antibody can be stored at 4°C – check the manufacturer's recommendation.

FAQs

Q1. What biomolecules can I label?

Lightning-Link® technology works by targeting free amine groups on your target, meaning it can be used to label most biomolecules.

As the protocols provided were optimised for labeling IgGs, we would recommend you adjust the amount of material you add to the Lightning-Link® vial to allow for molecular weight difference. This should be done without changing the volume added to the vial, as this could affect the conjugation efficiency.

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.

As a rough guideline, we would recommend changing the amount of material proportionally to the size difference with IgGs. An average IgG is about 160kD, therefore for a target that is ½ the size of an antibody (about 80kD), add ½ as much to the vial. Please note this is only a guideline and the best amount for your assay should be determined experimentally; our 3x10µg kits enable you to do this using small amounts of material and therefore at a low cost.

Calculate the amount of your sample you will need to add with our concentration calculator available at:

<https://www.innovabiosciences.com/innova/concentration-calculations.html>

For additional information please see our FAQs page:

<https://www.innovabiosciences.com/faqs/antibody-labeling-faqs.html>

My buffer doesn't fit the requirements

If your antibody buffer is not compatible with our kits, we have developed the AbSelect™ purification kit range that allows you to quickly and simply purify your antibody and is fully compatible with the Lightning-Link® kits.

The appropriate kit to use depends on your particular sample (species, buffer, contaminants, volume,...). We have designed a handy flow chart on the AbSelect™ webpage to help you select a kit, visit:

https://www.innovabiosciences.com/images/stories/innova/pdfs/AbSelect_Purification_Flowchart.pdf

Please consult the kit protocols to see the antibody amount/volume suitable for each kit.

If your antibody is already purified but its concentration is too low, you can concentrate it by using our Antibody Concentration and Clean Up Kit (product code 860-0010). This kit can also be used to remove low molecular weight contaminants such as azide, Tris or glycine by carrying out a buffer exchange into the buffer supplied in the kit, which is fully compatible with Lightning-Link®.

If your antibody contains BSA, you can now use our BSA removal kit to purify your antibody in a few simple steps. Please note this kit will also enable you to concentrate your antibody.

NB: All the AbSelect kits will ONLY work with antibodies. They will not purify other molecules. The only exception is the concentration and clean up kit (861-0010), which will work with other molecules greater than 10kD.