



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 20

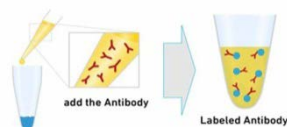
15/08/2016

Introduction

Lightning-Link® conjugation technology works by targeting amine groups (e.g. lysines) and is widely used to label antibodies.

The Lightning-Link® HRP conjugation kit allows HRP conjugations to be set up in *less than 30 seconds*, simply by adding a solution of the antibody to a lyophilized 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 antibody conjugation procedures, Lightning-Link® technology can be used to label both small (e.g. 10µg) 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%.

Directly labeled primary antibodies are advantageous as they eliminate the need for secondary reagents in immunoassay procedures, thus removing a tedious extra cycle of incubation and wash steps in applications such as ELISA and Western blotting.

Lightning-Link® technology can also be used to label proteins, peptides and other biomolecules (See the 'What can I label using Lightning-Link®?' section of this protocol for further information).

Kit contents and shipping conditions

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

1 vial of LL-modifier reagent

1 vial of LL-quencher reagent

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 after initial thawing 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 ¹	✗
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.

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 for 1:4 Ab:HRP	Amount of antibody for 1:1 Ab:HRP	Maximum Antibody Conjugation volume
701-0030	10µg	40µg	10µl
701-0000 701-0010	100µg	400µg	100µl
701-0002 701-0003	1mg	4mg	1ml

701-0004	5mg	20mg	5ml
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Superior conjugates are normally generated with a 1:4 Ab to HRP ratio. However good quality conjugates are still generated using a 1:1 Ab to HRP ratio.

Antibodies less than 1mg/ml can still be used to generate good conjugates provided the maximum conjugation volume is not exceeded. Adding less than the recommended maximum amount of antibody may result in unbound label post conjugation. This excess label will be deactivated by the quencher and removed during the first wash step of any application. We would recommend that antibodies below 0.5mg/ml are concentrated prior to use.

Please contact our technical support team for more advice.

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 lyophilized 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 room temperature overnight, as the longer incubation time has no negative effect on the conjugate.
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.

Storage of conjugates

Your HRP conjugate can be stored at 4°C for up to 18 months. For longer storage the conjugate can be stored at -20°C with a cryoprotectant such as 50% glycerol.

Our LifeXtend™ HRP conjugate stabilizer/diluent (product code 901-0005) is a proprietary multi-component reagent system that protects antibody-HRP conjugates thus ensuring the best possible performance in experiments performed at room temperature.

The best storage conditions for any particular conjugate must be determined by experimentation.

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What can I label using Lightning-Link®?

Lightning-Link® technology works by targeting free amine groups. It can be used to label antibodies, peptides, proteins and other molecules with free amine groups.

The protocol provided here is optimized for labeling IgGs, we would recommend when labeling other molecules you visit our dedicated alternative applications page, www.innovabiosciences.com/innova/advanced-users-guide.html

our Lightning-Link FAQ page for antibody related questions

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

or contact our technical support team.

technical.enquiries@innovabiosciences.com

Our custom conjugation service team will also be happy to generate your conjugate for you. Please contact our sales team for a quote.

info@innovabiosciences.com

What if my buffer doesn't fit the requirements?

The AbSelect™ purification kit range allows you to quickly and simply purify your antibody using our conjugation friendly buffers.

The appropriate kit to use depends on your particular sample. We have designed a handy flow chart on the AbSelect™ webpage to help you select the best kit;

<https://www.innovabiosciences.com/images/stories/innova/pdfs/abselect%20purification%20flowchart.pdf>

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 861-0010). This kit can also be used to remove low molecular weight contaminants such as azide, Tris or glycine.

If your antibody contains BSA, you can use our BSA removal kit (product code 820-0100) to purify your antibody in one simple step.

Our AbSelect™ kits are fully compatible with Lightning-Link® and are designed to work with IgG antibodies. The only exception is the Antibody Concentration and Clean Up Kit (product code 861-0010) which will work with any molecule greater than 10kDa.

I have a question regarding my conjugation

Please contact our technical support team at technical.enquiries@innovabiosciences.com if you require any further information or help about our product.