INTRODUCTION

Lightning-Link® conjugation technology works by targeting amine groups (e.g. lysines) and is widely used to label antibodies. It can also be used to label proteins, peptides and other biomolecules that contain available amine groups.

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).

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. 10 µg) 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.

KIT CONTENTS

- 1 x vial AbSelect™ BSA Removal Buffer
- 1 x vial AbSelect™ Re-suspension Buffer
- 1 vial 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.

CLEAN UP THE ANTIBODY (IF REQUIRED)

The antibody storage buffer and concentration of the antibody should be checked prior to conjugation. Use the guidelines below to ensure that your antibody is compatible with Lightning-Link®.

If your antibody is not compatible with Lightning-Link®, we offer a range of AbSelect™ purification kits enabling you to concentrate your antibody and/or perform a buffer exchange.

We have included an AbSelect™ BSA Removal kit within this sample kit in case clean-up is required. The AbSelect™ BSA Removal kit is a simple 1 step, 10-minute method which can be used to remove the contaminants listed in Table 1 from your antibody buffer; it can also be used to concentrate your antibody.

BUFFER CONSIDERATIONS

If the buffer contains substances which require removal, a buffer exchange should be performed prior to conjugation using one of our AbSelect™ Purification kits. Table 1 details contaminating substances, and states whether the included AbSelect™ BSA Removal Kit can remove these. Proceed with the AbSelect™ BSA Removal if applicable. If no clean-up is required proceed to ‘Setting up your conjugation reaction’. If a buffer exchange is required but the AbSelect™ BSA Removal kit is not suitable, contact our Technical Support Team before continuing.

<table>
<thead>
<tr>
<th>Buffer Components</th>
<th>Lightning-Link® conjugation kit can tolerate?</th>
<th>Can be resolved using BSA Removal kit?</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 6.5-8.5</td>
<td>✗</td>
<td>N/A</td>
</tr>
<tr>
<td>Amine free buffer (e.g. MES, MOPS, HEPES, PBS)</td>
<td>✗</td>
<td>N/A</td>
</tr>
<tr>
<td>Non-buffering salts (e.g. sodium chloride)</td>
<td>✗</td>
<td>N/A</td>
</tr>
<tr>
<td>Chelating agents (e.g. EDTA)</td>
<td>✗</td>
<td>N/A</td>
</tr>
<tr>
<td>Sugars</td>
<td>✗</td>
<td>N/A</td>
</tr>
<tr>
<td>Glycerol²</td>
<td>&lt;50%</td>
<td>&lt;20%</td>
</tr>
<tr>
<td>Thiomersal / Thimerosal</td>
<td>✗</td>
<td>✓</td>
</tr>
<tr>
<td>Merthiolate</td>
<td>✗</td>
<td>✓</td>
</tr>
<tr>
<td>Sodium Azide</td>
<td>✗</td>
<td>✓</td>
</tr>
<tr>
<td>BSA⁴</td>
<td>&lt;0.1%</td>
<td>✓</td>
</tr>
<tr>
<td>Gelatin³</td>
<td>&lt;0.1%</td>
<td>✗</td>
</tr>
<tr>
<td>Tris</td>
<td>&lt;50mM</td>
<td>✓</td>
</tr>
<tr>
<td>Glycine</td>
<td>✗</td>
<td>✓</td>
</tr>
<tr>
<td>Proclin</td>
<td>✗</td>
<td>✓</td>
</tr>
<tr>
<td>Borate buffer</td>
<td>✗</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Nucleophilic components
(Primary amines e.g. amino acids or ethanolamine and thiols e.g. mercaptoethanol or DTT)

¹ The AbSelect™ BSA Removal Kit is effective with buffers between pH 6.0 and 8.0.
² If your antibody contains glycerol at a higher concentration than 20%, dilute to reduce the glycerol content to below the threshold and then continue with the purification.

Figure 1. Lightning-Link® antibody conjugation

![Antibody](image1.png)

[Image of antibody and labeled antibody]
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.

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

Please note that when using the AbSelect™ BSA Removal Kit, 50µg of antibody is the lower limit for seeing a clearly visible pellet, and therefore is the minimum amount of antibody which should be purified using this kit.

### AMOUNT AND VOLUME OF ANTIBODY

<table>
<thead>
<tr>
<th>Product Code</th>
<th>Amount of antibody for 1:4 Ab:HRP</th>
<th>Amount of antibody for 1:1 Ab:HRP</th>
<th>Maximum Antibody Conjugation volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>701/820</td>
<td>10µg</td>
<td>40µg</td>
<td>10µL</td>
</tr>
</tbody>
</table>

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

**Antibody clean up (if required)**

1. Warm the AbSelect™ BSA Removal Buffer by placing the tube in warm water (not warmer than 40°C) for 10 minutes, then shaking to re-dissolve the contents. Once dissolved, maintain the tube at −22°C to prevent any crystal formation before use.
2. For every 100µl of antibody to be treated, add 80µl of the AbSelect™ BSA Removal Buffer directly to the antibody solution.
3. Mix and incubate for 5 minutes at room temperature.
4. Spin the sample in a bench top micro-centrifuge, at a recommended maximum speed of 13,000g for 5 minutes.* Position the Eppendorf tube in the centrifuge in such a manner that you know where any pellet will be located. We routinely position the hinge of the Eppendorf tube at the outside edge of the rotor.
5. Remove the sample from the centrifuge, taking care not to dislodge the small pellet at the bottom of the tube.** Remove the supernatant. The supernatant can be kept on ice until a positive outcome is confirmed.
6. Re-suspend the pellet using the AbSelect™ Re-suspension buffer provided, or another buffer suitable for the labeling process.

*The required spin time will vary depending on buffer composition and speed. The speed should not exceed 13,000g.

** If, after centrifugation, the supernatant appears cloudy and slightly viscous, a precipitate may have formed rather than a pellet. If a pellet cannot be seen, add 10% volume of water, incubate for a further 5 minutes, and centrifuge as before. If a pellet still cannot be seen, add 10% volume of AbSelect™ BSA Removal Buffer and centrifuge again. In the absence of a pellet at this stage, please contact our Technical Support Team before continuing.

### 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 labeled. 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 (20-29°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.

### TECHNICAL SUPPORT

For technical enquiries get in touch with our technical support team at: www.expedeon.com/contact/

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