Lightning-Link® conjugation kits: consistent performance on a broad scale

application note

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Introduction:
Overcoming typical conjugation issues

Finding the right combination of antibody and label of interest constitutes a significant challenge for many researchers when selecting conjugated primary antibodies. Unable to solve this challenge, researchers may be forced to compromise on antibody quality or switch to alternative approaches: generating a suitable conjugated antibody in-house, using a secondary antibody or changing aspects of experimental design, such as the multiplex panel composition. All these approaches have their limitations: in-house conjugation is time-consuming and requires expertise; secondary antibodies add extra complexity; continuous changes in experimental design typically result in additional time and costs spent on finding and revalidating reagents.

Still, conjugating primary antibodies in-house may seem an attractive alternative for several reasons, including the flexibility to combine your preferred antibodies with any label. However, traditional conjugation methods have complex protocols and usually require a certain level of expertise, thereby taking up valuable lab time and resulting in antibody loss. Furthermore, in-house conjugation may not produce consistent results during assay scale-up, such as transitioning from a proof-of-principle experiment to screening. Importantly, the quality of antibodies used for conjugation remains critical for achieving reproducible results on any experimental scale.

Our innovative Lighting-Link® labeling technology enables the direct labeling of antibodies or proteins, providing a straightforward solution to traditional conjugation issues. Lighting-Link kits don't require a post-conjugation purification step, eliminating the risk of antibody loss. More importantly, these kits can generate reproducible conjugates on any scale of your experiment and are, therefore, efficient across the whole length of your drug discovery program.

Lighting-Link kits can be easily combined with our conjugation-ready recombinant primary antibodies and antibody pairs, giving you the flexibility to create an antibody-label combination of your choice. Recombinant antibodies provide exceptional reproducibility and security of long-term supply, while conjugation-ready formulations are free of BSA, glycerol, and sodium azide, making them ideal for antibody labeling.

In this application note, we assess the reproducibility of the results delivered by four popular Lightning-Link conjugation kits across different batches and on the various conjugation scale ranging from 10 µg to 100 mg of antibody.

The principles of Lightning-Link® antibody labeling technology

Lightning-Link® antibody labeling technology uses a simple process whereby the antibody to be labeled is added into a vial of a lyophilized mixture containing the desired label (Figure 1). The conjugated antibody is then ready to use without the need for post-conjugation purification steps, removing the risk of antibody loss.

The advantages of this method include reproducibility and scalability, with some kits using as little as 10 µg of antibody and scaling up to 100 mg of antibody, and the ability to form antibody-label conjugates at a neutral pH. The simplicity of the antibody labeling process minimizes the amount of hands-on time to 30 seconds, while a wide choice of kits (over 50 in total) enables the flexibility to create the desired antibody-label combination suitable for your experimental setup.

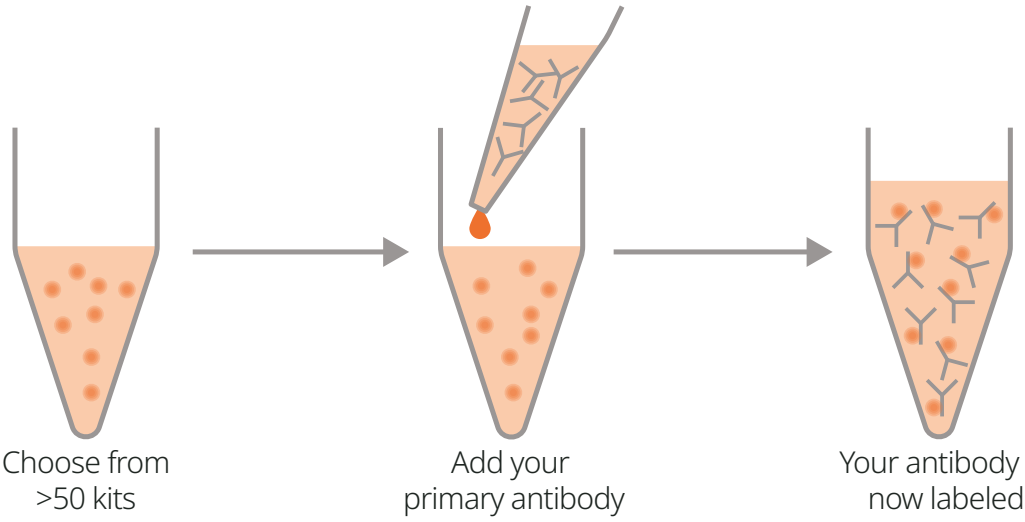

Figure 1. Diagram illustrating the simple Lightning-Link® protocol.

Materials and methods

Lightning-Link® conjugation kits:

— HRP Conjugation Kit - Lightning-Link® (ab102890)
— Alkaline phosphatase Conjugation Kit - Lightning-Link® (ab102850)
— Biotin Conjugation Kit (Fast, Type A) - Lightning-Link® (ab201795)
— Fluorescein Conjugation Kit (Fast) - Lightning-Link® (ab188285)

Tecan microplate reader

Instrumental procedure

For all four kits, samples were handled according to the protocol booklet recommendations. The standard curves were prepared based on 4- or 5-point dilution series.

For HRP and Alkaline phosphatase conjugation kits, each vial was conjugated to Goat anti-Rabbit IgG overnight; the conjugates were then diluted to 160 ng/mL or 227 ng/mL, respectively, added to a Goat anti-Rabbit IgG coated plate and mixed with Rabbit IgG ranging from 250 ng/mL to 15.6 ng/mL (Figure 2A-B, 3B), or from 150 ng/mL to 1.9ng/mL (Figure 3A). For the Biotin kit, each vial was conjugated to HRP for 15 minutes, and the conjugates were then diluted to 1.14 µg/mL, added to a Goat anti-Rabbit IgG coated plate, and mixed with Rabbit IgG-streptavidin ranging from 2.50 µg/mL to 0.156 µg/mL. For the Fluorescein kit, each vial was conjugated to Fluorescein for 15 minutes; the three conjugates were then serially diluted from 22.7 µg/mL to 0.356 µg/mL and added to a Rabbit IgG coated plate.

The signals were detected using a microplate reader from Tecan. The HRP and Alkaline Phosphatase signals were detected by reading at A405 nm after adding ABTS substrate and hydrogen peroxide for HRP and Biotin kits and pNPP substrate for Alkaline phosphatase kit. For the Fluorescein kit, the fluorescence intensity of the conjugates was measured at Ex: 490 nm and Em: 535 nm.

Results:
Batch-to-batch consistency

Our conjugation kits manufacturing sites are ISO 9001:2015 certified, and standardized and quantitative QC tests are routinely performed on all our batches to guarantee lot-to-lot reproducibility.

Figure 2 demonstrates the batch-to-batch consistency of up to four batches for the following Lightning-Link conjugation kits: HRP (A), Alkaline phosphatase (B), Biotin (C), and Fluorescein (D) when conjugating each kit to 100 µg of antibody.

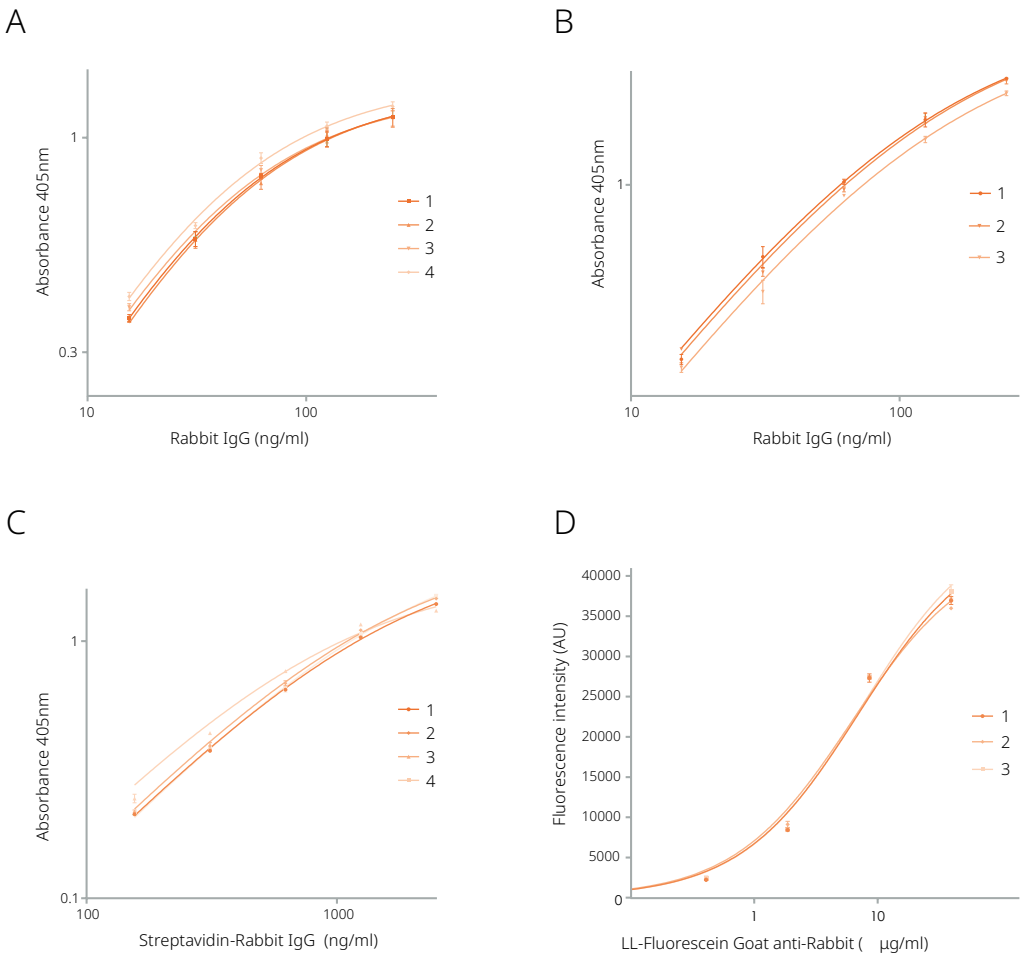

Figure 2. Batch-to-batch consistency of four different Lightning-Link conjugation kits: HRP (A), Alkaline phosphatase (B), Biotin (C), and Fluorescein (D) was tested in a sandwich (A-C) or direct (D) ELISA. Following the protocol booklet recommendations, each 100 µg vial was conjugated to 100 µg of Goat anti Rabbit IgG overnight (A-B), 100 µg of HRP for 15 minutes (C), or Fluorescein for 15 minutes (D).

Batch-to-batch consistency when scaling up and down

Our conjugation kits are available in a range of pack sizes, enabling fast and easy conjugation from 10 µg up to 100 mg of antibody.

Since the labeling process remains unaltered, the performance of antibody conjugates prepared at a small scale is identical to that prepared at a large scale. Figure 3 shows the consistent performance of the same four Lightning-Link kits on an experimental scale ranging from 10 µg to 100 mg of antibody.

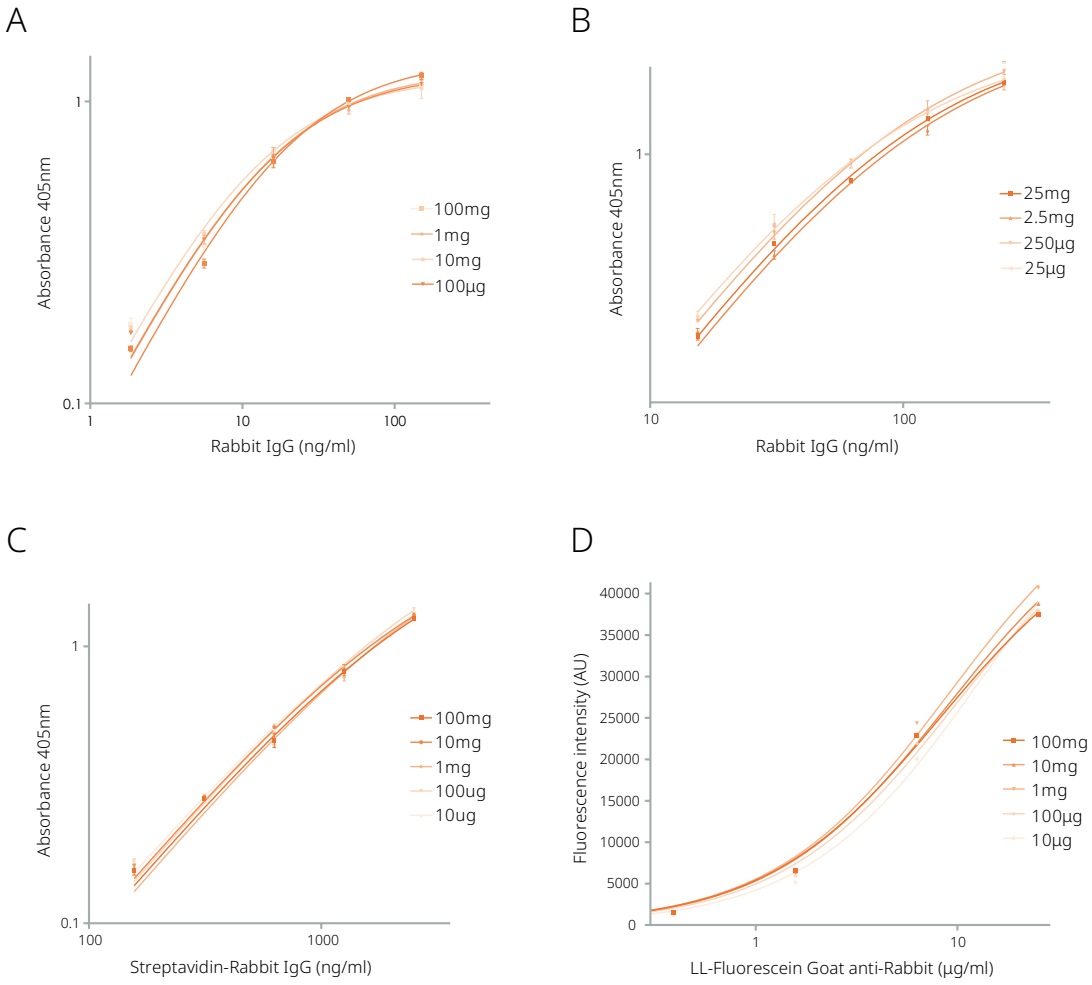

Figure 3. Consistency of conjugation scale-up ranging from 10 µg to 100 mg of antibody using HRP (A), Alkaline Phosphatase (B), Biotin (C), and Fluorescein (D) Lightning-Link® conjugation kits. Following the protocol booklet recommendations, vials were conjugated overnight to Goat anti-Rabbit IgG (A, B) or for 15 minutes to HRP(C) or fluorescein (D).

Conclusion

Lightning-Link® conjugation kits offer an excellent batch-to-batch consistency on the broad conjugation scale ranging from 10 µg to 100 mg of antibody while allowing quick and easy conjugation and the flexibility to use the desired label. The consistency of kit performance ensures that you can easily scale up or down your experiment, achieving reproducible results across the whole drug discovery pipeline. Furthermore, these kits can easily be combined with conjugation-ready recombinant antibodies with unrivaled reproducibility and guaranteed long-term supply, providing extra confidence in long-term results across the whole pipeline.