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# CHROMALINK® ONE-SHOT™ ANTIBODY BIOTINYLATION KIT

**SKU:** B-9007-009K


## DESCRIPTION

The ChromaLINK One-Shot Antibody Biotinylation Kit provides convenient, consistent, and measurable biotinylation of 100 µg of antibody. Each kit contains ChromaLINK Biotin, which incorporates a novel UV-traceable chromophore in the linker arm to enable reproducibility in your biotinylation process. Now you can measure the degree of biotinylation in minutes, not hours, without the standard curves required for the HABA/Avidin and Fluoro-Reporter assays. The ChromaLINK One-Shot kit contains everything needed to to biotinylate your antibody: buffers, reagents, desalting columns, and an easy-to-follow protocol and online biotin incorporation calculator.

## SPECIFICATIONS

| | |
|---|---|
| **Reactivity** | Amine |

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| | |
|---|---|
| **Unit Size** | 1 Kit |
| **Storage Instructions** | 2° – 8°C — Do Not Freeze |
| **Applications** | In Situ Proximity Ligation |
| **Conjugate** | Antibody |
| **Label** | biotin |

# KIT COMPONENTS

- ChromaLINK Biotin (6.49 µg)
- 1X Modification Buffer (1.5 mL)
- 1X PBS (2 x 1.5 mL)
- 2 mL Collection Tube (4)
- 0.5 mL Zeba™ Desalting Column (2)
- Biotinylated Bovine IgG Control (100 µg)
- 1M Tris HCl (1.5 mL)
- Anhydrous DMF (1.5 mL)

# TECHNICAL INFORMATION

## A. Product Description

The ChromaLINK Biotin One-Shot Antibody Labeling Kit is designed to biotinylate a single 100 microgram quantity of antibody in about 2.0 hours. The ChromaLINK One-Shot kit relies on a UV-traceable linker called ChromaLINK Biotin to label the antibody (**Figure 1**). This unique labeling reagent contains an aromatic, water-soluble N-hydroxy-succinimidyl ester functional group. (**a**) that efficiently modifies antibody lysine residues under mild phosphate buffer conditions. The reagent also possesses an embedded bis-aryl hydrazone structure (**b**) which forms the linker's UV-traceable chromophore. The absorbance signature provided by the chromophore enables rapid and non-destructive quantification of incorporated biotin. (**c**). The linker also features a long PEG3 spacer (**d**) which preserves streptavidin/biotin affinity and helps maintain antibody solubility.

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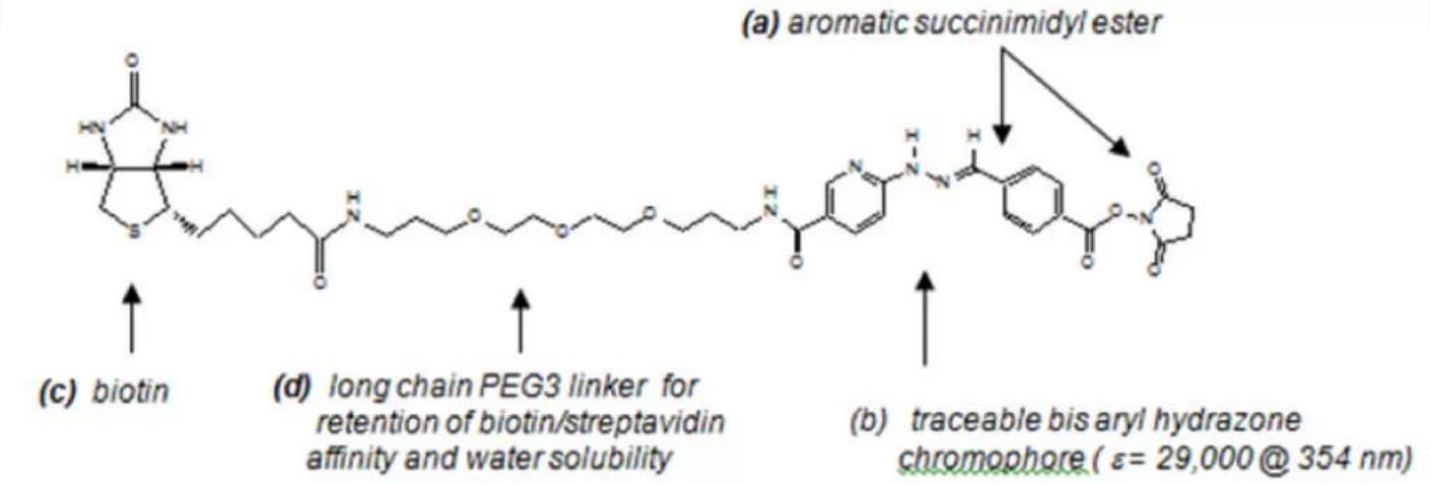


**Figure 1. Structure of ChromaLINK Biotin C38H50N8O10S; MW 810.92.**

## B. Features and Benefits

The ChromaLINK Biotin One-Shot Kit is a simple, cost-effective way of incorporating a readily verifiable amount of biotin into a single 100 µg quantity of antibody. The kit conveniently features a pre-weighed amount of ChromaLINK Biotin reagent that is readily resuspended directly into the antibody solution. ChromaLINK Biotin can be used to label a variety of different antibodies, including mammalian IgG (monoclonal or polyclonal) and/or avian IgY. Biotin incorporation is rapidly determined by means of a simple, non-destructive UV-scan (220–400 nm) of the sample after removal of excess labeling reagent. The kit features high antibody recovery (60–90 µg) and a consistent level of biotin incorporation (3–8 biotin molecules per antibody molecule) when used as directed.

## C. ChromaLINK One-Shot Process Diagram

The ChromaLINK Biotin One-Shot antibody labeling procedure is illustrated in **Figure 2**.

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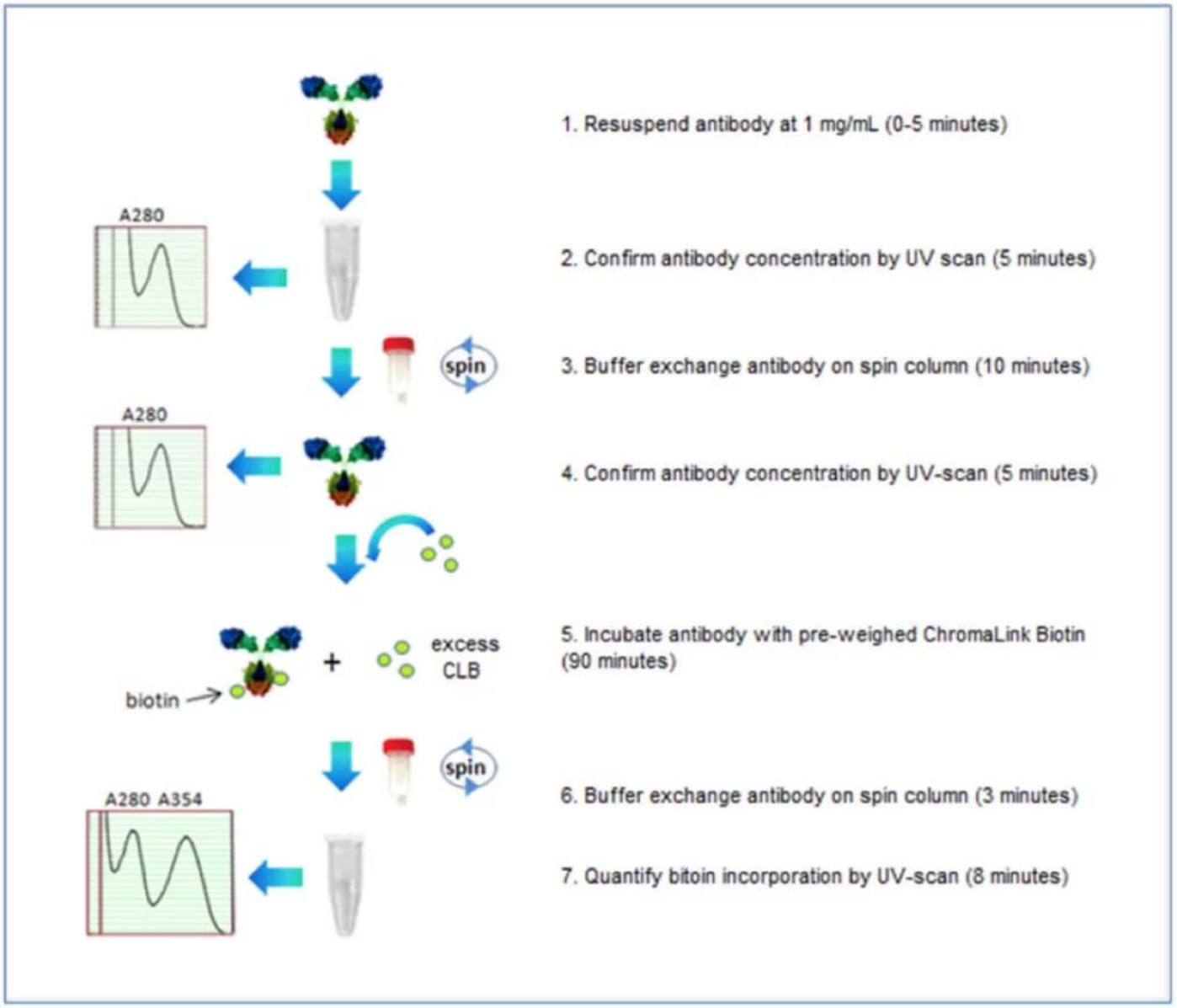


**Figure 2. ChromaLINK Biotin One-Shot antibody labeling procedure.**

## D. Process Summary

1. *Sample Preparation*: bring antibody to 1 mg/mL in 100 µL buffer
2. *1st Sample Analysis*: confirm antibody concentration using spectrophotometer
3. *1st Buffer Exchange*: equilibrate spin columns and buffer exchange antibody
4. *2nd Sample Analysis*: reconfirm antibody concentration using spectrophotometer
5. *Biotinylation*: label antibody with ChromaLINK Biotin
6. *2nd Buffer Exchange:* remove excess labeling reagent
7. *Quantify Biotin Incorporation*: measure biotin incorporation using spectrophotometer

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## E. Important Labeling Parameters

The ChromaLINK Biotin One-Shot Antibody Labeling Kit is designed to biotinylate a single 100 µg quantity of antibody resuspended in 100 µL buffer as indicated in Table 1.

| Initial Antibody Concentration | Intitial Mass of Antibody | Initial Antibody Volume |
|---|---|---|
| 1.0 ± 0.1 mg/ml | 100 ± 10 µg | 100 ± 5 µl |

**Table 1. Initial conditions required for the ChromaLINK Biotin One-Shot labeling procedure.**

This kit provides a consistent and reliable degree of biotinylation by controlling the following reaction variables:

- Fixed antibody mass (100 µg) and volume (100 µL)
- Optimized reaction buffer
- Controlled and fixed reaction time (90 min)
- Consistent reaction stoichiometry (12X mole-equivalents) (12-fold molar excess of biotin reagent)

Critical to consistent biotin incorporation is the ability of a user to accurately determine the antibody's **initial protein concentration** in a non-destructive manner that allows full or nearly full recovery of the precious sample. The ChromaLINK One-Shot procedure recommends using a scan (220–400 nm) on a spectrophotometer rather than the traditional A280 to estimate antibody concentration. The rationale for using a scan rather than a single wavelength (A280) is that many commercial antibodies contain preservatives and/or other additives that mask or distort the intrinsic A280 of a sample. The presence of an additive can make it more difficult or sometimes impossible to accurately estimate protein concentration via A280. A scan however contains greater **spectral information**, often revealing the presence of spectrum-altering additives that are known to interfere with the accurate estimate of protein concentration. Altered spectra are a warning that the A280 of the sample will not yield an accurate estimate of protein concentration. Commonly used additives include preservatives such as sodium azide, thimerosal, protein stabilizers such as BSA or gelatin and/or small molecule additives such as glycine or trehalose.

# CITATIONS

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# DOCUMENTS

- User Guide
- Safety Data Sheet
- ChromaLINK Biotin Conjugation Calculator
- Troubleshooting Guide – Bioconjugation
- Download CoA
- Datasheet

# GALLERY IMAGES


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