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# CHROMALINK® DIGOXIGENIN ONE-SHOT™ ANTIBODY LABELING KIT

**SKU:** B-9014-009K

## DESCRIPTION

The ChromaLINK Digoxigenin One-Shot Antibody Labeling Kit provides convenient, consistent, and measurable digoxigenin labeling of 100 µg of antibody. Each kit contains ChromaLINK Digoxigenin, which incorporates a novel UV-traceable chromophore in the linker arm to enable reproducibility in your antibody labeling process. With a simple, non-destructive UV scan you can now quantify digoxigenin incorporation to ensure reproducible incorporation of the optimal number of haptens per antibody. Each ChromaLINK One-Shot kit contains everything needed to to label your antibody: buffers, reagents, desalting columns, and an easy-to-follow protocol and online DIG incorporation calculator.

## SPECIFICATIONS

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

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

# KIT COMPONENTS

- Sulfo ChromaLINK® Digoxigenin (11 µg)
- 1X Modification Buffer (1.5 mL)
- 1X PBS (1.5 mL)
- Anhydrous DMF (1.5 mL)
- 2 mL Collection Tube (4)
- 0.5 mL Zeba™ Desalting Column (2)
- DIG-labeled Bovine IgG Control (100 µg)
- 1M Tris HCl (1.5 mL)

# TECHNICAL INFORMATION

## Product Description

The ChromaLINK Digoxigenin One-Shot antibody labeling kit is specifically designed to incorporate a digoxigenin label into a single 100 microgram quantity of antibody resuspended in 100 µl of buffer. This kit comes complete with all the necessary components to label and rapidly quantify incorporated digoxigenin from a small quantity of antibody in about 90 minutes.

Digoxigenin has long been used as an alternative hapten reporter system to the streptavidin/biotin system (1). This system is based on a steroid isolated from the blossoms and leaves of the plant *Digitalis purpurea*, the only known natural source of digoxigenin. High affinity antibodies raised against digoxigenin thus exhibit virtually no cross-reaction with other biological molecules found in higher organisms. Both the specificity and sensitivity of this “parallel” hapten-reporter system often gives digoxigenin a major advantage over biotin-based detection systems. In more recent times, this reporter system has also been used in conjunction with the biotin-streptavidin system to detect multiplexed antigens (2). Unlike the biotin/streptavidin system, the digoxigenin label has never had a method for directly quantifying incorporation, until now.

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The labeling process used in the ChromaLINK Digoxigenin One-Shot kit is based on a patented UV-traceable linker called Sulfo-ChromaLINK Digoxigenin (**Figure 1**). This advanced digoxigenin labeling reagent contains an aromatic water-soluble N-hydroxy-sulfosuccinimidyl ester functional group (**a**), which efficiently modifies protein lysine residues in a phosphate buffer system. The linker also possesses an embedded bis-aryl hydrazone structure (**b**), which forms the compound's UV-traceable chromophore. This traceable signature enables the non-destructive and direct quantification of digoxigenin (**c**), attached to the antibody. These diverse chemical elements are linked together through a long-chain PEG3 spacer (**d**), which preserves antibody-digoxigenin binding affinity while simultaneously maintaining antibody solubility.

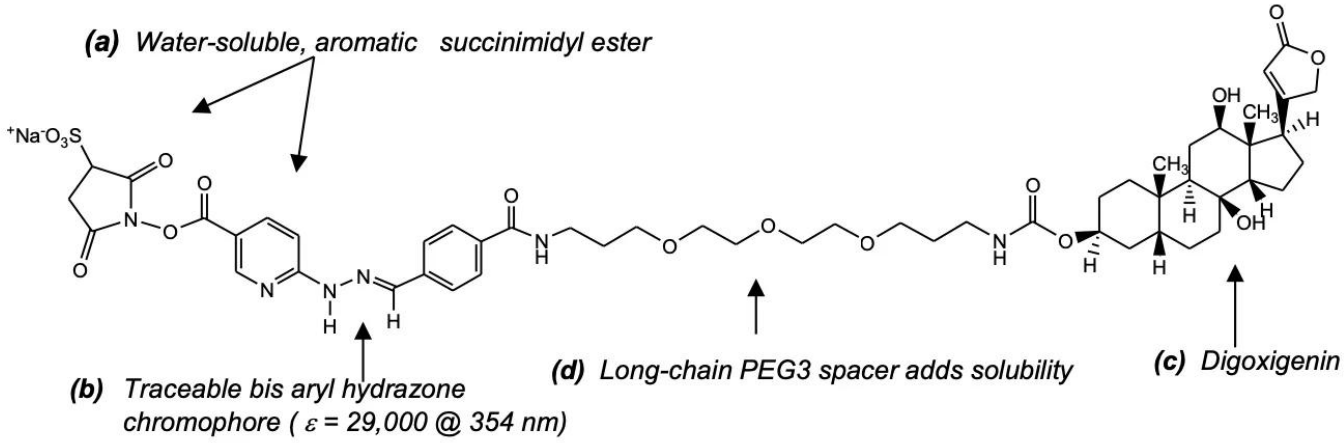


**Figure 1**: Structure of ChromaLINK Digoxigenin C52H67N6NaO17S; M.W. 1103.17

## Benefits and Features

The ChromaLINK Digoxigenin One-Shot kit is a convenient, cost-effective way of incorporating digoxigenin into a small quantity (100 µg) of an antibody. After incorporation, digoxigenin is rapidly quantified using a non-destructive UV-scan (220-400 nm). The kit features high antibody recoveries (50-80%) with consistent digoxigenin incorporation. The degree of incorporation or molar substitution ratio (MSR) ranges from 2-8 digoxigenin molecules per antibody when used as directed.

The ChromaLINK Digoxigenin One-Shot kit can be used to label a diverse group of antibodies including all mammalian IgG, IgE, and avian IgY molecules. The ChromaLINK Digoxigenin One-Shot kit offers a robust method of both labeling and quantifying incorporated digoxigenin with a simple protocol. This "double-feature" provides researchers with greater confidence, consistency and reproducibility in all downstream applications.

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## Procedure Diagram

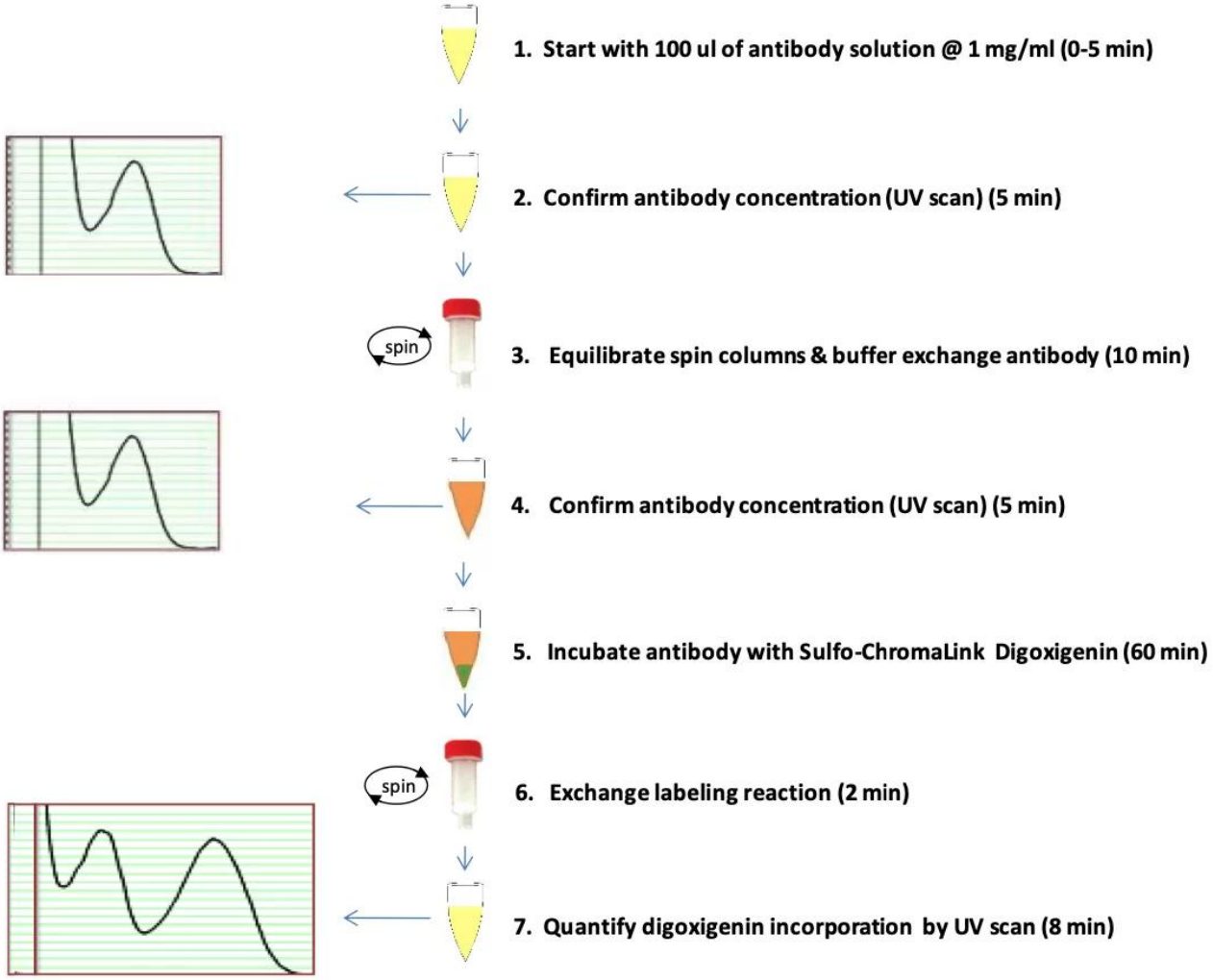


**Figure 2.** Illustration of the ChromaLINK Digoxigenin One-Shot antibody labeling procedure.

## Process Summary

**Sample Preparation:** adjust antibody to 1 mg/ml in 100 µl buffer

**Sample Analysis:** confirm antibody concentration using a spectrophotometer

**First Buffer Exchange:** equilibrate spin columns & buffer exchange antibody

**Sample Analysis:** reconfirm recovered antibody concentration on a spectrophotometer

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**Digoxigenin Labeling:** label antibody with ChromaLINK Digoxigenin linker

**Second Buffer Exchange:** remove excess labeling reagent with spin column

**Digoxigenin Incorporation:** quantify incorporation using a spectrophotometer

## Important Labeling Parameters

The ChromaLINK Digoxigenin One-Shot antibody labeling kit is specifically designed to incorporate digoxigenin into a single 100 microgram quantity of antibody resuspended in 100 µl of a suitable buffer as indicated in **Table 1**.

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

**Table 1.** Initial starting conditions required for the ChromaLINK Digoxigenin One-Shot procedure.

The kit provides consistent and reliable incorporation of digoxigenin by controlling the following reaction variables:

- Initial antibody mass (100 µg) and volume (100 µl)
- Reaction buffer composition
- Reaction time (60 min)
- Reaction stoichiometry (15-fold mole-equivalents of linker)

Critical to digoxigenin incorporation is the ability to accurately determine an antibody's initial concentration in a non-destructive manner with full recovery of the sample. The ChromaLINK Digoxigenin One-Shot procedure employs a spectral scan (220-400 nm) rather than a single wavelength measurement @ 280 nm to accomplish this task. Commercial antibodies often contain preservatives or other additives capable of masking or distorting the intrinsic UV spectrum of a sample. As a consequence, additives make it more difficult to **accurately** estimate protein concentration using a single wavelength (i.e. A280) measurement. A scan however, provides greater information and assurance that the antibody's concentration is correct since a spectrum often reveals the presence of interfering additives.

Commonly used additives may include preservatives such as sodium azide, thimerosal, protein stabilizers such as *BSA or *gelatin, or small molecule additives such as glycine. *Note-protein

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*carriers such as BSA or gelatin must be removed before labeling can proceed*.

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# DOCUMENTS

- User Guide
- Safety Data Sheet
- Bioconjugation White Paper
- B-9014 – ChromaLINK Digoxigenin Conjugation Calculator
- Troubleshooting Guide – Bioconjugation
- Download CoA
- Datasheet

# GALLERY IMAGES

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