

# ChromaLINK® Digoxigenin One-Shot™ Antibody Labeling Kit

Cat. No. B-9014-009K

Storage 2°–8°C—Do Not Freeze.

The ChromaLINK Digoxigenin One-Shot Antibody Labeling Kit requires 100 µg of antibody at a concentration of 1.0 mg/ml. The antibody buffer should be free of carrier proteins such as BSA or gelatin.

## Description

The ChromaLINK Digoxigenin One-Shot Antibody Labeling Kit contains all the necessary reagents and components to label a single 100 µg quantity of antibody with digoxigenin. Based on **SoluLINK® bioconjugation technology**, it allows any antibody to be conjugated and purified within 90 minutes, involving just 30 minutes of hands-on time (Figure 1). Because the ChromaLINK Digoxigenin One-Shot Antibody Labeling Kit uses a UV-traceable linker for antibody labeling, digoxigenin incorporation can rapidly be determined by a simple, non-destructive UV measurement (280 and 354 nm). The kit features high antibody recovery (50–80 µg) and a consistent level of digoxigenin incorporation (2–8 digoxigenin molecules per antibody) for reproducible results.

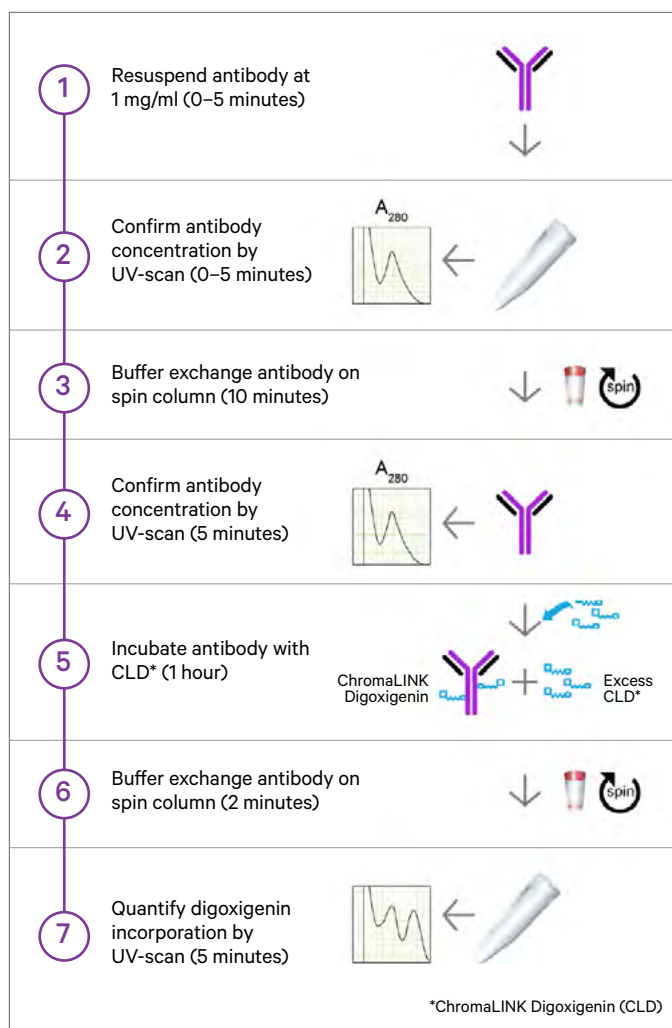


Figure 4 ChromaLINK Digoxigenin One-Shot Antibody Labeling Kit workflow.

## Kit Components

Component	Amount
Sulfo ChromaLINK Digoxigenin	11 µg
1X Modification Buffer	1.5 ml
1X PBS	1.5 ml
0.5 ml Thermo Scientific™ Zeba™ Column	2
2 ml Collection Tube	4
DIG-labeled Bovine IgG Control	100 µg
Anhydrous DMF	1.5 ml
1M Tris-HCl	1.5 ml

## Protocol

### A. Prepare antibody

Use the included 1X Modification Buffer to dissolve lyophilized antibody or dilute aqueous antibody solution to a concentration of 1.0 mg/ml. If the antibody is at less than 1.0 mg/ml, it must be concentrated prior to beginning. Centrifugal diafiltration apparatus are available which accommodate up to 500 µl of dilute antibody solution. Choose a molecular weight cutoff in the 10–30 kDa range and follow the manufacturer's instructions for concentrating dilute protein samples.

### B. Buffer exchange antibody

Once the antibody is confirmed to be at a concentration of  $1.0 \pm 0.1$  mg/ml and a volume of 100 µl, buffer exchange the sample as follows:

1. Prepare two Zeba spin columns (red caps) by twisting off the bottom closures and loosening the caps one-half turn (do not remove completely).
2. Place each spin column into a 2 ml collection tube.
3. Place the spin columns opposite each other in the microcentrifuge and spin at 1,500 x g for 1 minute to remove storage solution.

**Important:** Ensure the centrifuge is set to "g" or RCF rather than RPM in all centrifugation steps.

4. Remove the columns and discard the flow-through from the bottom of the collection tubes. Do not discard the collection tubes.
5. Using a lab marker, place a vertical mark on the side of each spin column where the compacted resin is slanted upward.
6. Mark one spin column cap with the letter "A" and the other spin column cap with the letter "B".
7. Slowly add 300 µl of 1X Modification Buffer to the top of column A, and 300 µl of 1X PBS to the top of column B. When applying the buffer or sample, do not disturb the resin bed with the pipet tip.
8. Loosely re-cap the columns.

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9. Place the spin columns back into their collection tubes and orient the vertical marks facing outward (away from the center of the rotor) in the centrifuge.
10. Centrifuge at 1,500 x g for 1 minute to remove buffer.
11. Repeat steps 7 through 10 two additional times, discarding the flow-through each time.
12. After the last spin, transfer the equilibrated spin column A into a new 2 ml collection tube.
13. Discard the buffer from the column B collection tube and add 300 µl of 1X PBS to the column. Loosely re-cap and set this column aside on the bench; it will be used later to buffer exchange the DIG-labeled antibody.
14. Slowly load the antibody sample (100 µl at 1.0 mg/ml) onto spin column A.
15. Loosely re-cap column A, orient it in the centrifuge with the vertical line facing outward, and centrifuge it at 1,500 x g for 2 minutes.

**Note:** Use a microcentrifuge tube containing water as a balance during this step. Do not use column B as a balance.

16. Transfer the desalted antibody solution from the column A collection tube to a labeled microcentrifuge tube. Retain the column A assembly to use as a balance later.
17. Measure the antibody concentration using a conventional UV-Vis spectrophotometer or a NanoDrop™ spectrophotometer to confirm antibody recovery.
18. If the antibody concentration is >0.8 mg/ml and >90 µl, proceed to step C.

**Note:** If the amount of antibody recovered is below this volume and/or concentration, obtain additional antibody before proceeding.

#### C. Label antibody with digoxigenin

1. Briefly centrifuge the vial containing ChromaLINK Digoxigenin at 10,000 x g to ensure the pellet is at the bottom. A very small pellet should be visible.
2. Add 5.0 µl of anhydrous DMF directly to the pellet and pipet up and down until completely dissolved.
3. Add the desalted antibody solution directly to the dissolved ChromaLINK Digoxigenin reagent and mix by pipetting up and down several times, then gently vortexing.
4. Incubate the reaction for 60 minutes at room temperature.
5. When the incubation is complete, quench the reaction by adding 10 µl of 1M Tris HCl.

6. Centrifuge the quenched reaction at 1,500 x g for 30 seconds then set the reaction aside.
7. Place the previously hydrated spin column B assembly containing 300 µl of 1X PBS (section B, step 13) into the centrifuge. Orient the assembly with the vertical mark facing outward.
8. Add 300 µl of water to spin column A and place it opposite column B in the centrifuge.
9. Centrifuge at 1,500 x g for 1 minute. Discard the flow-through from each collection tube.
10. Transfer column B to a new 2 ml collection tube. Proceed immediately to step D.

#### D. Buffer exchange digoxigenin-labeled antibody

1. Add the entire contents of the quenched digoxigenin labeling reaction (section C, step 6) to the center of column B. Re-cap the column loosely.
2. Apply 100 µl of water to spin column A and re-cap loosely.
3. Orient column B in the centrifuge with the vertical mark facing outward and place column A across from it.
4. Centrifuge at 1,500 x g for 2 minutes.
5. Transfer the digoxigenin-labeled antibody from the bottom of collection tube B to a labeled storage tube.

#### E. Measure the digoxigenin MSR

The digoxigenin molar substitution ratio (MSR, or number of digoxigenin labels attached per antibody) is determined by measuring the DIG-labeled sample in a conventional UV-Vis or Thermo Scientific NanoDrop™ Spectrophotometer. Follow the instructions below for the type of instrument available.

##### UV-Vis spectrophotometer MSR procedure

1. Program the spectrophotometer to scan from 220–400 nm. If scanning is not available, measure the 280 nm and 354 nm absorbance values individually.
2. Using a clean semi-micro quartz cuvette (≤100 µl), blank the instrument using 1X PBS.
3. Discard the blank solution and dry the cuvette.
4. Transfer the digoxigenin-labeled antibody sample to the cuvette and scan.
5. Record the 280 nm and 354 nm absorbance values from the scan.

**Important:** Recover the DIG-labeled antibody sample from the cuvette.

6. Enter the  $A_{280}$  and  $A_{354}$  values into the [ChromaLINK Digoxigenin MSR Calculator](#), along with the antibody E1% value (Table 1) and molecular weight. The calculator will display the digoxigenin molar substitution ratio (MSR).

Antibody Source	Antibody E1% (1-cm path)
Human IgG	13.60
Human IgE	15.30
Rabbit IgG	13.50
Donkey IgG	15.00
Horse IgG	15.00
Mouse IgG	14.00
Rat IgG	14.00
Bovine IgG	12.40
Goat IgG	13.60
Avian IgY	12.76

Table 1. Mass extinction coefficients (E1%, 280 nm) for antibodies derived from different host species. The E1% is used to calculate antibody concentration and represents the  $A_{280}$  of a 10 mg/ml solution measured in a 1-cm pathlength cuvette. Most antibodies of the IgG isotype have a molecular weight of 150,000 Da.

#### NanoDrop spectrophotometer MSR procedure

1. Initialize the instrument with water, if required (NanoDrop 1000 only).
2. Select the Protein  $A_{280}$  menu option. Do not use the UV-Vis function to determine the MSR.
 

**Important:** Deselect the 340 nm normalization option, if available. If left selected, it will automatically zero the baseline at 340 nm and significantly lower the MSR value.
3. In the “Sample Type” window, select “Other Protein E1%” from the pull-down menu. Enter the appropriate E1% value (refer to Table 1 above) corresponding to the antibody host species.
4. Blank the instrument with 2  $\mu$ l of 1X PBS.
5. Click the “Measure” icon to verify that a flat baseline has been obtained. Clean the pedestal and repeat the blank procedure until a flat baseline is observed, if necessary.
6. Place 2  $\mu$ l of digoxigenin-labeled antibody on the pedestal and click the “Measure” icon. The spectrum (220–350 nm) should appear.
7. Record the  $A_{280}$  directly from the absorbance window.
8. Obtain the  $A_{354}$  from the scan by manually entering 354 into the  $\lambda$  (wavelength) window and recording the value displayed.
9. Enter the  $A_{280}$  and  $A_{354}$  values into the [ChromaLINK Digoxigenin MSR Calculator](#), along with the antibody E1% (refer to Table 1) and molecular weight. The calculator will display the digoxigenin MSR.

#### Using the DIG-labeled bovine IgG control to validate MSR measurements

The ChromaLINK Digoxigenin One-Shot Antibody Labeling Kit includes a digoxigenin-labeled antibody control. This consists of lyophilized digoxigenin-labeled bovine IgG at a known digoxigenin molar substitution ratio. The control can be used to check the accuracy of a spectrophotometer, and to validate MSR measurements. To use the DIG-labeled bovine IgG control, add 100  $\mu$ l of water and pipet the solution up and down for at least 1 minute to fully dissolve the antibody to 1.0 mg/ml. Centrifuge the vial for 30 seconds at 1,500  $\times$  g, then scan the sample in a conventional UV-Vis or NanoDrop spectrophotometer as described in section E. The digoxigenin-labeled IgG control has an MSR value of  $3.5 \pm 1.5$ .

#### Storage

The digoxigenin-labeled antibody should be stored at 2–8°C. A bacteriostatic agent such as 0.05% sodium azide or 0.01% thimerosal may be added to prevent microbial growth and extend shelf-life.

#### Application Notes

##### [Troubleshooting Guide](#)