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USER GUIDE

ChromaLINK® One-Shot™ Antibody Biotinylation Kit

Together we breakthrough™

Cat. No. B-9007-009

Storage 2°–8°C—Do Not Freeze.

The ChromaLINK One-Shot Antibody Biotinylation 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 One-Shot Antibody Biotinylation Kit contains all the necessary reagents and components to biotinylate a single 100 µg quantity of antibody. Based on SoluLINK® bioconjugation technology, it allows any antibody to be biotinylated and purified within 2 hours, involving just 30 minutes of hands-on time (Figure 1). Because the ChromaLINK One-Shot Antibody Biotinylation Kit uses a UV-traceable linker for antibody labeling, biotin incorporation can rapidly be determined by a simple, non-destructive UV measurement (280 and 354 nm). The kit features high antibody recovery (60–90 µg) and a consistent level of biotin incorporation (3–8 biotin molecules per antibody) for reproducible results.

Kit Components

Component	Amount
ChromaLINK Biotin	6.49 µg
1X Modification Buffer	1.5 ml
1X PBS	1.5 ml
2 ml Collection Tube	4
0.5 ml Thermo Scientific™ Zeba™ Desalting Column	2
Biotinylated Bovine IgG Control	100 µg
1M Tris HCl	1.5 ml
Anhydrous DMF	1.5 ml

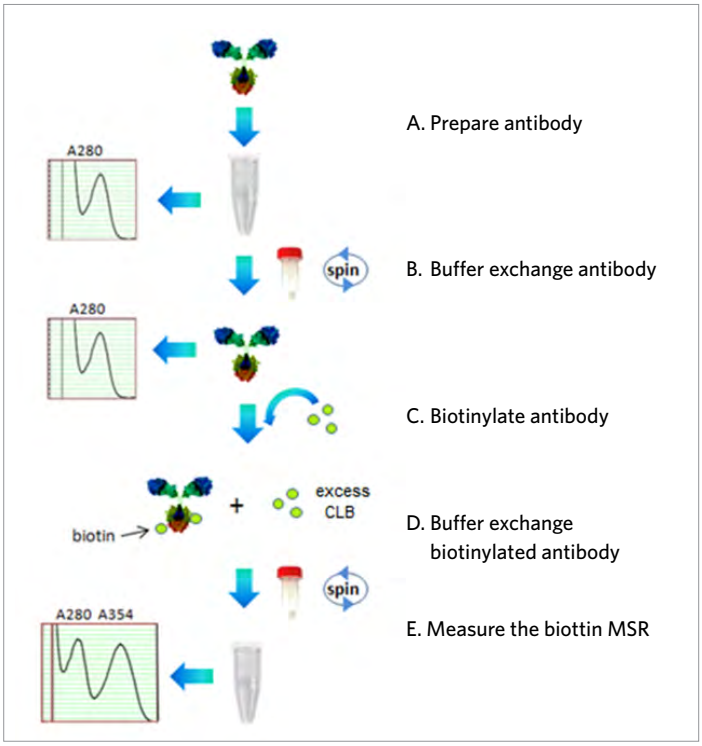

Figure 1. ChromaLINK One-Shot antibody biotinylation workflow.

Protocol

A. Prepare antibody

Use the 1X Modification Buffer (included) 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 ± 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). Place each spin column into a 2 ml collection tube.
2. Mark the top of one cap with the letter "A" and the other cap with the letter "B" using a lab marker.
3. Place a vertical mark on the side of each spin column using a lab marker.
4. Place each assembly into the centrifuge and orient the vertical mark on the spin columns facing outward (away from the center of the rotor).
5. Centrifuge at 1,500 x g for 1 minute. Discard the flow-through, then place the columns back into the empty collection tubes.

   Important: Ensure the centrifuge is set to "g" or RCF rather than RPM in all centrifugation steps.
6. 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. Loosely re-cap the columns.
7. Place each assembly in the centrifuge and orient the vertical marks facing outward.
8. Centrifuge at 1,500 x g for 1 minute. Discard the flow-through from the bottom of the collection tubes.

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9. Repeat steps 6 through 8 two additional times, discarding the flow-through each time.
10. Add 300 µl of 1X PBS to the top of column B, re-cap loosely, and set this spin column aside on the bench. It will be used later to buffer exchange the biotinylated antibody.
11. Transfer spin column A to a new collection tube. Add 100 µl of antibody at 1.0 mg/ml to the top of column A and loosely re-cap.
12. Place column A in the centrifuge and orient the vertical mark facing outward. Balance the rotor with a microcentrifuge tube containing water (do not use column B as a balance).
13. Centrifuge at 1,500 x g for 2 minutes.
14. Transfer the antibody solution from the bottom of the collection tube to a labeled microcentrifuge tube. Set the column A assembly aside to use as a balance later.
15. Measure the antibody concentration using a conventional UV-Vis spectrophotometer or a NanoDrop™ spectrophotometer to confirm antibody recovery.
16. If the antibody concentration is >0.8 mg/ml and >90 µl, proceed to step C.

    Important: If the recovered antibody is below this volume and/or concentration, obtain additional antibody before proceeding.

C. Biotinylate antibody

1. Briefly centrifuge the vial containing ChromaLINK Biotin 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 to the pellet and pipet up and down until completely dissolved.
3. Add the desalted antibody solution directly to the vial of resuspended ChromaLINK Biotin.
4. Mix the solution by pipetting up and down several times, then gently vortexing.
5. Incubate the reaction for 90 minutes at room temperature.
6. When the incubation is complete, quench the reaction by adding 10 µl of 1 M Tris HCl. Set the quenched reaction aside.
7. Place the previously equilibrated column B assembly containing 300 µl of 1X PBS (Section B, step 10) in the centrifuge and orient the vertical mark facing outward.
8. Add 300 µl of water to the used column A assembly (Section B, step 14) to use as a balance tube opposite column B.
9. Centrifuge at 1,500 x g for 1 minute. Discard the flow-through from the bottom of each collection tube.

   Important: Ensure the centrifuge is set to "g" or RCF rather than RPM in all centrifugation steps.
10. Transfer column B to a new 2 ml collection tube. Proceed immediately to section D.

D. Buffer exchange biotinylated antibody

1. Add the quenched biotinylation reaction (Section C, step 6) to the top of column B. Loosely re-cap the column.
2. Add 100 µl of water to column A, re-cap loosely, and use as a balance.
3. Orient column B with the vertical mark facing outward and spin at 1,500 x g for 2 minutes.
4. Transfer the biotinylated antibody from the bottom of collection tube B to a labeled storage tube.

E. Measure the biotin MSR

The biotin molar substitution ratio (MSR, or number of biotins attached per antibody) is determined by measuring the sample using a conventional UV-Vis or NanoDrop spectrophotometer. Follow the instructions below for the type of instrument available.

Conventional 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 biotinylated antibody sample to the cuvette and scan.
5. Record the 280 nm and 354 nm absorbance values from the scan.

   Important: Recover the biotinylated antibody sample from the cuvette.
6. Enter the A_{280} and A_{354} values into the ChromaLINK Biotin MSR Calculator, along with the antibody E1% value (Table 1) and molecular weight. The calculator will display the biotin MSR.

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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 µl 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 µl of biotinylated 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 λ (wavelength) window and recording the value displayed.
9. Enter the A_{280} and A_{354} values into the ChromaLINK Biotin MSR Calculator, along with the antibody E1% (refer to Table 1) and molecular weight. The calculator will display the biotin MSR.

Using the biotinylated IgG control to validate MSR measurements

The ChromaLINK One-Shot Antibody Biotinylation Kit includes a biotinylated antibody control. This consists of lyophilized biotinylated bovine IgG at a known biotin molar substitution ratio. The control can be used to check the accuracy of a spectrophotometer, and to validate MSR measurements.

To use the biotinylated IgG control, add 100 µl 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 x g, then scan the sample in a conventional UV-Vis or NanoDrop spectrophotometer as described in section E. The biotinylated IgG control has an MSR value of 4.5 ± 1.5.

Storage

The biotinylated 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

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