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- Trockeneiszuschlag
- Gefahrgutzuschlag
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USER GUIDE

ChromaLINK® Biotin Protein Labeling Kit

vector LABORATORIES

Together we breakthrough™

Cat. No. B-9007-105

Storage 2°–8°C—Do Not Freeze.

The ChromaLINK Biotin Protein Labeling Kit requires 25–1,000 µg of protein at a concentration of 0.25–10 mg/ml in a volume of 100 µl. The kit is suitable for biotinylating proteins with a molecular weight range of 20,000–950,000 Daltons. Proteins must be free of carrier proteins such as BSA or gelatin for successful biotinylation.

Description

The ChromaLINK Biotin Protein Labeling Kit contains all the necessary reagents and components to biotinylate and purify up to five proteins with molecular weights ranging from 20–950 kDa. The biotinylation process takes approximately 3 hours, involving just 45 minutes of hands-on time. The scale of each protein labeling reaction is flexible, and can accommodate as little as 25 µg to as much as 1 mg of protein using a fixed volume of 100 µl. Because the ChromaLINK Biotin Protein Labeling Kit uses a UV-traceable linker for biotinylation, biotin incorporation can rapidly be determined by means of a simple, non-destructive UV reading at 280 and 354 nm. The kit provides high protein yields (70–80%) and a consistent level of biotin incorporation (3–8 biotin molecules per protein) for reproducible results.

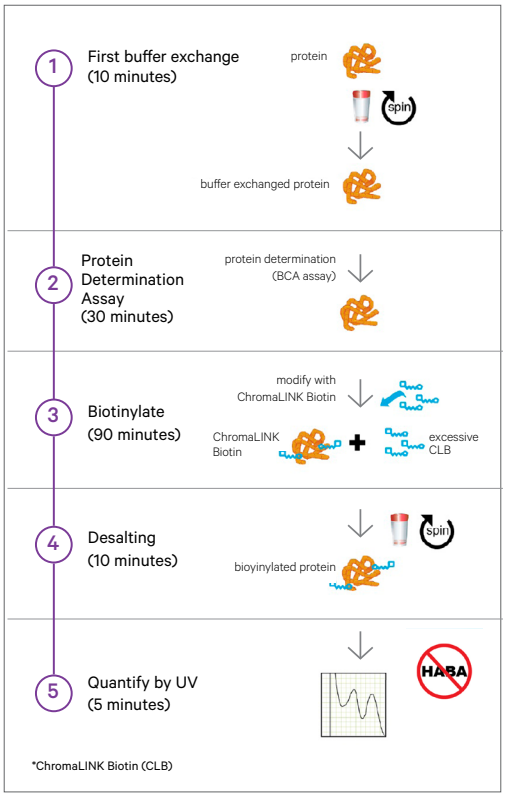

Figure 3 ChromaLINK Biotin Labeling Kit workflow.

Kit Components

Component	Amount
ChromaLINK Biotin	5 x 0.5 mg
10X Modification Buffer	2 x 1.5 ml
10X PBS	2 x 1.5 ml
0.5 ml Thermo Scientific™ Zeba™ Column	12
2 ml Collection Tube	24
1.5 ml Storage Tube	12
Bovine IgG Control	0.5 mg
Biotinylated Bovine IgG Control	0.5 mg
Anhydrous DMF	1.5 ml

Protocol

Each biotin labeling reaction performed with the ChromaLINK Biotin Protein Labeling Kit uses a fixed volume of protein solution (100 µl) and either 10 or 20 mole equivalents of ChromaLINK Biotin reagent depending on the protein concentration and molecular weight. The ChromaLINK Biotin Protein Labeling Calculator is used to calculate the volume of ChromaLINK Biotin to add to the protein. The molecular weight and concentration of the protein to be labeled should be within the range specified in Table 1.

Protein Concentration Range	Protein Mass Range	Molecular Weight Range	Volume Protein Required
0.25–10 mg/ml	25–1000 µg	20–950 kDa	100 µl

Table 1. Range of acceptable labeling conditions for ChromaLINK Biotin Protein Labeling Kit.

A. Prepare buffers and protein sample

Dilute 10X Modification Buffer to 1X concentration using ultrapure water. Dissolve lyophilized protein samples, or dilute aqueous protein samples to a concentration of 0.25–10 mg/ml in a total volume of 100 µl using 1X Modification Buffer, if required. Dilute 10X PBS to 1X concentration using ultrapure water. Approximately 1.5 ml of each 1X buffer is required per biotinylation reaction.

B. Buffer exchange protein

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).

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5. Centrifuge at 1,500 x g for 1 minute. Discard the flow-through from the bottom of the collection tubes, then place the columns back into the empty 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 into the centrifuge and orient the vertical marks facing outward.
8. Centrifuge at 1,500 x g for 1 minute. Discard the flow-through.
9. Repeat steps 6 through 8 two additional times.
10. Add 300 µl of 1X PBS to the top of column B, re-cap loosely, and set this column aside on the bench. It will be used later to buffer exchange the biotinylated antibody.
11. Transfer spin column A to a new 2 ml collection tube. Slowly add 100 µl of protein at 0.25–10 mg/ml to the top of column A without disturbing the resin 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 desalted protein solution from the bottom of collection tube A to a labeled 1.5 ml storage tube while measuring the volume with a P-200 pipet. Set the column A assembly aside to use as a balance later.

C. Measure protein concentration

After buffer exchange, measure the protein concentration using a conventional UV-Vis spectrophotometer or a NanoDrop™ if the mass extinction coefficient (E1%) is known. Alternatively, a BCA or Bradford protein assay may be used to determine protein concentration if the E1% is unknown. The biotinylation reaction requires 100 ± 10 µl at a protein concentration between 0.25–10 mg/ml.

D. Biotinylate protein

After confirming the protein volume and concentration, the protein is ready to be biotinylated.

1. Prepare a stock solution of ChromaLINK Biotin by dissolving one 0.5 mg vial in 100 µl of anhydrous DMF. Ensure the reagent is fully dissolved by pipetting up and down several times and vortexing until no solids remain.
2. Using the ChromaLINK Biotin Protein Labeling Calculator, input the name, molecular weight, and concentration of the protein. The calculator will display the volume of ChromaLINK Biotin needed for the biotinylation reaction.
3. Add the indicated volume of ChromaLINK Biotin working solution to the protein and immediately pipet up and down to mix.

   Note: If the volume of ChromaLINK Biotin working solution required is less than 2 µl, a dilution of the solution can be made and the volume added to the protein increased proportionally. Do not exceed 5 µl of ChromaLINK Biotin working solution in the protein sample, however, as >5% anhydrous DMF can denature some proteins.

4. Allow the reaction to incubate for 2 hours at room temperature. As the reaction proceeds, some cloudiness may be observed (usually after 15–30 minutes) in more concentrated protein samples due to partial hydrolysis of the concentrated NHS ester.
5. Five minutes prior to the end of the labeling reaction, transfer spin column B (equilibrated in PBS during section B, step 10) into the centrifuge. Add 300 µl of water to the used column A and place it opposite column B to serve as a balance tube.
6. Orient column B with the vertical mark facing outward and centrifuge the columns at 1,500 x g for 1 minute. Discard the flow-through from each collection tube.

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

7. Transfer spin column B to a new 2 ml collection tube and immediately proceed to section E.

E. Buffer exchange biotinylated protein

1. Transfer the completed biotinylation reaction to the top of Zeba column B. Orient the column in the centrifuge with the vertical line facing outward.
2. Add 100 µl of water to column A and place it opposite column B.
3. Spin at 1,500 x g for 2 minutes to collect the buffer-exchanged biotinylated protein sample.

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

4. Transfer the biotinylated protein from the bottom of collection tube B to a labeled storage tube. The biotin molar substitution ratio (MSR) of the labeled protein can now be determined.

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F. Determine biotin MSR

The biotin molar substitution ratio (MSR) can be determined using a conventional UV-Vis spectrophotometer, UV-Vis plate reader, or a NanoDrop spectrophotometer. Refer to Table 2 for each instrument's specific requirements and limitations. Generally, for 1-cm pathlength cuvettes and 96-well plates, the optimal protein concentration for scanning is 0.5–1.0 mg/ml. More concentrated protein samples (2–10 mg/ml) will require dilution prior to scanning. If using a plate, ensure it is UV-transparent and has a flat bottom. When using a NanoDrop spectrophotometer, some protein samples will be too dilute for accurate UV measurements due to the short pathlength of this instrument.

Instrument	Protein Concentration	Compatibility
UV-Vis Spectrophottometer	< 0.5 mg/ml 1 mg/ml 2–10 mg/ml	✓ ✓ ✓*
Plate Reader	< 0.5 mg/ml 1 mg/ml 2–10 mg/ml	N.R. ✓ ✓*
NanoDrop Spectrophotometer	< 0.5 mg/ml 1 mg/ml 2–10 mg/ml	N.R. ✓ ✓*

Table 2. Instrument-specific sample requirements. N/R = not recommended, ✓ = compatible, ✓ = compatible but sample will require dilution.*

The biotin MSR can be determined using either the E1% method or the BCA method. The E1% method is used when the protein's E1% (mass extinction coefficient) is known. This is the A280 of a 1% (10 mg/ml) solution measured in a 1-cm pathlength cuvette. A list of published E1% values is provided in Table 3 for reference. If the E1% of a protein is unknown, the BCA method can be used.

Protein	E1%, 280 nm (1-cm pathlength)
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
Human IgA	12.60
Human IgM	11.80
Bovine Serum Albumin	6.70
Trypsin	16.00
Chymotrypsin	20.20
Ovalbumin	7.90
Alpha-Amylase	24.20

Table 3. Published E1% values for commonly biotinylatetd proteins.

E1% Method

1. Program a conventional UV-Vis spectrophotometer, plate reader, or NanoDrop to scan from 220–420 nm. If scanning is not available, read the sample at 280 and 354 nm.

   Note: If using a NanoDrop spectrophotometer, the UV-Vis module can be used for the scan. Ensure the baseline correction function is turned off during all measurements, if applicable.

2. Blank the spectrophotometer with 1X PBS. If using a standard spectrophotometer, a quartz 1-cm pathlength microcuvette is recommended.
3. Transfer a portion of the biotinylated protein sample to the cuvette, plate, or pedestal and scan from 220–420 nm. If the absorbance reading at 280 or 354 nm is out of range for the instrument, repeat using an aliquot diluted in 1X PBS.
4. Transfer the biotinylated protein back into its storage tube.
5. Record the 280 nm and 354 nm absorbance values from the scan. If using a NanoDrop, ensure the absorbance values are normalized to a 1-cm (10-mm) pathlength.
6. Calculate the biotin MSR using the E1% ChromaLINK Biotin MSR Calculator by inserting the name of the biotinylated protein, the protein molecular weight, the volume recovered from the biotinylation reaction, the 280 and 354 nm absorbance values, and the protein E1% value into the light green input fields.
7. The calculator will display the biotin MSR value.

BCA Method

1. Blank a spectrophotometer at 354 nm using 1X PBS.
2. Measure the 354 nm absorbance of the biotinylated protein and record the value. If using a NanoDrop, ensure the absorbance value is normalized to a 1-cm (10-mm) pathlength.
3. Measure the biotinylated protein concentration using a BCA or Bradford protein assay according to the manufacturer's instructions.
4. Calculate the biotin MSR using the BCA Assay ChromaLINK Biotin MSR Calculator by inserting the name of the biotinylated protein, the volume of biotinylated protein recovered from the labeling process, the molecular weight of the protein, the 354 nm absorbance, and the BCA (or Bradford) protein concentration.
5. The calculator will display the biotin MSR value.

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Using the Biotinylated Bovine IgG Control to validate MSR measurements

The ChromaLINK Biotin Protein Labeling Kit comes with a lyophilized, pre-biotinylated bovine IgG control. This can be used to validate MSR calculations and absorbance readings with a conventional UV-Vis spectrophotometer, plate reader, or NanoDrop spectrophotometer. Follow the instructions below to use the biotinylated bovine IgG control

1. Resuspend the biotinylated bovine IgG control to a concentration of 1.0 mg/ml by adding 500 µl of ultrapure water and pipetting up and down, then gently vortexing to mix.
2. Briefly spin the tube at 1,500 x g for 15 seconds.
3. Follow the instructions in section F for measuring MSR using the E1% method. Bovine IgG has an E1% of 12.40 and a molecular weight of 150,000 Da.
4. Enter the required information into the E1% ChromaLINK Biotin MSR Calculator to determine the biotin MSR.
5. The biotinylated bovine IgG control has an MSR value of 4.5 ± 1.5 biotin molecules per protein.

Biotinylating the bovine IgG control

The ChromaLINK Biotin Protein Labeling Kit comes with an unlabeled bovine IgG control that is ready for biotinylation. To use the control, simply resuspend the lyophilized bovine IgG in 500 µl of ultrapure water to obtain a 1.0 mg/ml protein solution, then proceed directly to the biotinylation reaction in section D. As the bovine IgG control has been pre-equilibrated in 1X Modification Buffer, no desalting is required prior to biotinylation. Bovine IgG has an E1% of 12.40 and a molecular weight of 150,000 Da. The MSR value obtained after biotinylation should be between 3–8 biotin molecules per protein.

Storage

Biotinylated proteins should be stored refrigerated at 2–8°C. For delicate proteins, a stabilizer such as BSA or gelatin may be added after the MSR and protein concentration have been determined. A bacteriostat such as 0.05% sodium azide or 0.01% thimerosal may be added to prevent microbial growth and extend the shelf life of the protein, if desired.

Application Notes

Troubleshooting Guide

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