

(More detailed instructions and troubleshooting available at www.prosci-inc.com)

Kit Components (Cat. No. PSI-4001)

Components	Size / Quantity	Storage Conditions
Sulfo-Redi-Pro™ Biotin	8.0µg / 1	Room Temperature
1x Modification Buffer (pH 7.4)	1.5mL / 1	Room Temperature
1x PBS (pH 7.2)	1.5mL / 1	Room Temperature
Collection tubes	1.5mL / 4	Room Temperature
Zeba™ Spin Columns	0.5mL / 2	Room Temperature
Biotinylated IgG Control	100µg / 1	Room Temperature
1M Tris•HCL (pH 8.9)	100µL / 1	Room Temperature

Additional Materials Required

UV-VIS or NanoDrop™ Spectrophotometer (capable of reading several wavelengths 100nm apart)
Semi-micro quartz cuvette (50-100 µL capacity) (not required with NanoDrop™)
Variable speed microcentrifuge (e.g. Eppendorf 5415D, IEC MicroMax or similar)
1.5 ml microfuge tubes, molecular grade water, P-10, P-200, P-1000 pipettes

Procedure

a. Sample Preparation

1. Adjust the antibody to a concentration of 1mg/mL in 100µL of buffer.
 - a. Lyophilized proteins, initial sample is:
 - i. 100µg/vial: Re-suspend in 100µL of the 1x Modification Buffer for a concentration of 1±0.1mg/mL.
 - ii. > 120µg/vial: Re-suspend in enough of the 1x Modification Buffer for a concentration of 1mg/mL. Aliquot 100µL to a new 1.5mL microfuge tube for conjugation. Store the remainder of the antibody.
 - iii. < 90µg/vial: We do not recommend using the Redi-Pro™ Biotin kit for samples than 90µg.
 - b. Proteins in solution, initial sample is at:
 - i. 1mg/mL: If the antibody is in >100µL PBS or TBS then proceed to Sample Analysis.
 - ii. > 1mg/mL: Adjust the concentration to 1mg/mL and 100µL by adding the 1x Modification Buffer. Transfer 100µL to a new microfuge tube and store the remainder of the antibody.
 - iii. < 1mg/mL: Concentrate the antibody to 1mg/mL and 100µL before proceeding to Sample Analysis.

b. Sample Analysis by UV-VIS Spectrophotometer:

1. Program a spectrophotometer to scan from 220-400nm (a 100nm range is OK, for example 270-370nm).
2. Blank the instrument using PBS. Discard the blank solution.
3. Transfer the antibody (100µL at 1mg/mL) to the cuvette and scan.
4. Record the A₂₈₀ from the scan. If the spectrophotometer can only read two points, record the A₃₅₄ as well.
5. Calculate the initial antibody concentration using the equation below.

Equation: $[A_{280} / E1\%] \times 10 \text{ mg/mL} = \text{protein concentration (mg/mL)}$

* E1% is the mass extinction coefficient, see table below:

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

6. Adjust the concentration to be 1±0.1mg/mL and 100µL by addition of the 1x Modification Buffer if necessary. *Note:* Sample should be as close to 100µg and 100µL as possible.

c. NanoDrop™ Spectrophotometer

1. Set up the machine to read A₂₈₀ (do not use the UV-VIS option). Do not normalize against 340nm.
2. Use the table above to enter the appropriate E1% value corresponding to you sample.
3. Blank the spectrophotometer using 2µL of PBS. Repeat until a flat baseline is obtained.
4. Use 2µL of the sample to measure the concentration. Wait until the spectrum (220-350nm) appears in the window.
5. Record your antibody concentration.
6. For more instructions, see the detailed protocol.

d. First Buffer Exchange (10 min)

1. Prepare the two spin columns provided by twisting off the bottom closures and loosening the red caps.
2. Place each spin column into separate 1.5 ml microcentrifuge collection tubes.
3. Spin both at 1500 x g for 1 minute to remove the storage solution from the resin.
4. Remove columns from the centrifuge and discard the solutions from the bottom of the collection tubes.
5. Using a marker pen, place a mark on the side of each spin column where the compacted resin is slanted upward.

6. Mark the top of one cap with the letter **A** and the other spin column cap with the letter **B**.
7. Add 300µL of the 1x Modification Buffer (pH 7.4) to the top of resin bed A and 300µL of the 1x PBS to the top of resin bed B. Loosely cap the lids.
8. Place the spin columns back into their used collection tubes, centrifuge at 1500 x g for 1 minute to remove the buffer. (Orient spin column with pen mark away from the center of the rotor.)
9. Repeat steps 7 and 8 two additional times, discarding the flow-through buffer each time.
10. After the last spin, transfer the equilibrated spin column A into a new 1.5mL collection tube.
11. Buffer exchange the antibody sample (100µL at 1mg/mL) by loading the contents to the top of the equilibrated spin column A.
12. Add 100µL of 1x PBS to the top of the equilibrated spin column B to use as a balance tube. Recap the lid loosely.
13. Centrifuge the columns at 1500 x g for 2 minutes to collect the eluate at the bottom of the collection tubes.
14. Remove the spin column tube assembly A containing the antibody sample from the centrifuge and set aside. Do not discard this eluate.
15. Remove the spin column tube assembly B from the centrifuge and discard the bottom eluate. Add an additional 300µL of 1x PBS to the top of the resin bed B to rehydrate the resin, cap and set aside for later use.

e. Sample Analysis (5 min)

1. Using a spectrophotometer or NanoDrop™ scan the buffer exchanged antibody sample from collection tube A to confirm the amount of recovered antibody.
2. Calculate the recovered antibody concentration as before.
3. If the antibody is at 1 ± 0.2 mg/mL in a volume of 100 ± 5 µL, proceed to the biotinylation procedure below. If the concentration is higher, dilute with 1x Modification Buffer. If it is lower, see the Troubleshooting Guide found in the detailed protocol

f. Biotinylation Procedure (60 min)

1. Transfer the buffer exchanged antibody solution from the bottom of collection tube A (100µL at 1mg/mL) directly to a vial of Sulfo-Redi-Pro™ Biotin labeling reagent.
2. Mix thoroughly by pipetting 10-20 times, vortex for a few seconds. Briefly centrifuge the contents of the tube (~5 sec) to collect the entire reaction mixture at the bottom of the tube.
3. Allow the reaction to proceed for 60 minutes at room temperature.
4. After the reaction is complete, quench the reaction by adding 10µL of 1M Tris (pH 8.7) and mix well. Set aside.
5. Place the B spin column assembly containing 300µL 1x PBS into the centrifuge. Loosely cap the lid.
6. Add 300µL molecular grade water to the previously used A spin column.
7. Centrifuge both at 1500 x g for 1 minute. Discard the flow through from each spin column assembly.
8. Transfer the B spin column only to a new 1.5mL collection tube.

g. Second Buffer Exchange (3 min)

1. Add the entire contents of the quenched biotinylation reaction above to the center of the compacted B resin spin column. Recap the column loosely.
2. Apply 100µL of molecular grade water to the center of spin column A. Recap loosely.
3. Spin both columns at 1500 x g for 2 minutes.
4. Transfer the biotinylated antibody (~100µL) from the bottom of collection tube B to a new 1.5mL microfuge tube and cap.

h. Determining Biotin Incorporation using UV-VIS Spectrophotometer (10 min)

1. Follow steps 1-3 in section 'b' above.
2. Record A_{280} and A_{354} from the spectrum.
3. Transfer the sample to a clean tube for storage and label appropriately. Store at 4°C.
4. Input the A_{280} and A_{354} values along with the corresponding E1% into the Biotin MSR calculator (available at www.prosci-inc.com) to automatically calculate the biotin molar substitution ratio (MSR).
 - a. Note: Typical MSR values range from 3-8 biotins per antibody. The amount of biotinylated antibody (mass) recovered can range from 50 to 100µg depending on the exact starting mass and type of antibody being labeled.

i. Determining Biotin Incorporation using NanoDrop™ Spectrophotometer (10 min)

1. Follow instructions in section 'c' above to prepare the NanoDrop™.
2. Use 2µL of the sample to measure the concentration. Wait until the spectrum (220-350nm) appears in the window.
3. Record the absorbance at A_{280} from the λ absorbance window.
4. Record the absorbance at A_{354} by typing '354' in the λ window.
5. Input the A_{280} and A_{354} values along with the corresponding E1% into the Biotin MSR calculator (available at www.prosci-inc.com) to automatically calculate the biotin molar substitution ratio (MSR).
 - a. Note: Typical MSR values range from 3-8 biotins per antibody. The amount of biotinylated antibody (mass) recovered can range from 50 to 100µg depending on the exact starting mass and type of antibody being labeled.

j. Biotinylated IgG Control

The Redi-Pro™ Antibody Biotinylation Kit comes complete with a biotinylated antibody control. This control consists of a lyophilized biotinylated bovine IgG at a precisely known biotin molar substitution ratio (see vial label for the value). This control is used to validate the accuracy of a given spectrophotometer and to validate MSR calculations.

1. Blank the spectrophotometer using 1x PBS following the instructions in section 'b' or use section 'c' for the NanoDrop™.
2. Re-suspend the control (lyophilized biotin-IgG, 100µg) using 100µL molecular grade water at 1.0mg/mL and close the lid.
3. Centrifuge the control very briefly at a low speed (30 sec at 1500 x g).
4. Remove an aliquot and scan the biotin labeled antibody.
5. Record A_{280} and A_{354} from the spectrum.
6. Input these values along with the E1% (12.40) and M.W. (150kDa) into the biotin MSR calculator to determine the biotin molar substitution ratio.
7. Confirm the value obtained with the lot specific MSR found on the product label.