FluoReporter® Mini-biotin-XX Protein Labeling Kit

Catalog no. F6347

Table 1. Contents and storage information.

Material	Amount	Storage	Stability
Biotin-XX, sulfosuccnimidyl ester, sodium salt (Component A)	5 vials	4°C	When stored as directed, the product is stable for 6 months.
Reaction tubes, 2 mL capacity, each containing a stir bar (Component B)	5 tubes		
Spin columns (Component C)	5 columns		
Collection tubes (Component D)	5 tubes		
Dialysis tubing, molecular weight cut-off of ~12,000–14,000 daltons (Component E)	5 pieces		
Purification resin (Component F)	10 mL		
Number of reactions: The FluoReporter® Mini-Biotin-XX Protein Labeling Kit contains sufficient reagents for five biotinylation reactions of 0.1–3 mg each.			

Introduction

The FluoReporter® Mini-Biotin-XX Protein Labeling Kit provides a method for efficiently biotinylating small amounts of antibodies or other proteins. The watersoluble biotin-XX sulfosuccinimidyl ester contained in this kit readily reacts with a protein's amines to yield a biotin moiety covalently attached via two aminohexanoic chains ("XX"). This 14-atom spacer has been shown to enhance the ability of the biotin moiety to bind to avidin's relatively deep binding sites. Also included are ready-to-use spin columns and narrow dialysis tubing with a molecular weight cut-off of ~12,000–14,000 daltons. The spin columns provide a convenient method of purifying the biotinylated protein from excess biotinylation reagents. Alternatively, for protein volumes larger than 0.5 mL, the researcher may choose to remove excess reagents by dialysis, thereby avoiding further dilution of the biotinylated protein.

Before Starting

Materials Required but Not Provided

- Phosphate-buffered saline (PBS) without Ca^{2+}/Mg^{2+}, pH 7.2 (Cat. no. 70013)
- 1 M sodium bicarbonate, ph 8.3–8.5

Preparing Sodium Bicarbonate Solution

To prepare 1 M sodium bicarbonate, dissolve 0.84 g of $NaHCO_3$ in 10 mL of distilled water. The pH of the solution should be about 8.3–8.5.

Experimental Protocols

Guidelines for Preparing the Antibody for Biotinylation

- The antibody must be purified from serum and other proteins before it is biotinylated.
- If the purified antibody is at a concentration of 0.5–3 mg/mL in a dilute buffer, such as 10–20 mM sodium phosphate buffer, then it may be used directly in the following protocols.
- If the purified antibody is in a buffer containing primary amines, such as ammonium ions, Tris, or glycine, then it must first be desalted by using one of the spin columns provided (for a sample volume of 50–250 µL, see steps 1.6–1.11) or by dialyzing in PBS (for a sample volume of 0.5–2 mL, see steps 2.7–2.8).
- The presence of low concentrations (less than 0.1%) of biocides, including sodium azide and thimerosal, does not significantly affect the biotinylation reaction.
- For biotinylating larger amounts of proteins (5–20 mg per reaction), we recommend the FluoReporter® Biotin-XX Protein Labeling Kit (F-2610).

Biotinylating 0.2 mL of 0.5–3 mg Antibody

1.1 Transfer 200 µL of a 0.5–3 mg/mL antibody solution to a 2 mL reaction tube containing a stir bar (Component B). Add one-tenth volume (20 µL) of a freshly prepared 1 M sodium bicarbonate solution.

1.2 Add 200 µL distilled water to one vial of biotin-XX, sulfosuccinimidyl ester, sodium salt (Component A). Pipet the contents of the vial up and down to completely dissolve. Because this reactive form of biotin-XX rapidly hydrolyzes in water, you must prepare the aqueous solution immediately before use.

1.3 Refer to Table 2 for the amount of reactive biotin-XX solution that should be added to different concentrations of antibody solution to achieve approximately 3–8 biotin molecules per IgG.

If necessary, you may carry out the biotinylation reaction with smaller volumes (50–200 µL) of antibody solution, but the amount of 1 M sodium bicarbonate and of biotin-XX solution must be reduced in proportion to the volume of antibody to maintain the appropriate concentrations of these reagents in the reaction mixture. The volume of reactive biotin-XX solution recommended in Table 2 for a given protein concentration (mg/mL) and protein solution volume (mL) should also be effective when biotinylating proteins other than antibodies, or immunoglobulin classes other than IgG.

Table 2. Amount of reactive biotin-XX solution to use with different concentrations of antibody (Ab) solution.

Concentration of Ab solution (mg/mL)	Volume of Ab solution (mL)	Amount of biotin-XX solution (µL)
0.5	0.2	2
1.0	0.2	3
1.5	0.2	4
2.0	0.2	5
2.5	0.2	6
3.0	0.2	7

1.4 While stirring, add the appropriate amount of reactive biotin-XX solution to the reaction tube containing the antibody and sodium bicarbonate. Mix thoroughly.

1.5 Stir the reaction mixture for 1–1.5 hours at room temperature.

1.6 While the labeling reaction is taking place, take one spin column (Component C) out of the refrigerator and remove the upper cap. Add purification resin (Component F) to the column until full (approximately 1.5 mL) and allow the resin to settle.

1.7 After the resin has settled, remove the bottom closure of the column and allow the column buffer to drain by gravity. Discard this flowthrough.

1.8 Place the spin column in a 2 mL collection tube (Component D) (note that this tube does not contain a stir bar) and centrifuge the column/tube for 3 minutes at 1,100 × g using a swinging bucket rotor. Discard the buffer in the collection tube and save the empty collection tube for step 1.10. The spin column is now ready for purifying the biotinylated antibody (or for desalting the antibody solution, see Guidelines Preparing the Antibody for Biotinylation).

1.9 After the biotinylation reaction has incubated for 1–1.5 hours, apply the entire reaction mixture to the center of the spin column. Allow the solution to absorb into the gel bed. If any precipitation has occurred during the biotinylation reaction, centrifuge the sample for 5 minutes in a microcentrifuge before loading the column; only the supernatant should be loaded onto the column. Note that if the volume of the reaction mixture exceeds 250 µL per spin column, this purification procedure will not adequately separate the biotinylated protein from free biotin.

1.10 Place the spin column in the empty collection tube and centrifuge for 5 minutes at 1,100 × g. To convert g-force into rpm, consult the conversion chart provided by the centrifuge manufacturer or use the following equation:

$$\text{Relative centrifugal force} = (1.12 \times 10^{-5})\,(\text{rpm})^2\,(\text{radius})$$

where the relative centrifugal force (RCF) = g-force; rpm = revolutions per minute; and radius = radius in centimeters measured from the center of the centrifuge spindle to the bottom of the rotor bucket.

1.11 The collection tube now contains the purified biotinylated antibody in approximately 200–250 µL PBS with 2 mM sodium azide (which was the buffer contained in the spin column initially). Discard the spin column.

1.12 Typically, about 70–80% of the antibody in the biotinylation reaction is recovered as biotinylated conjugate. Thus, the concentration of the biotinylated antibody solution can be approximated using the following equation:

$$\text{mg/mL biotinylated protein} = \frac{\text{mg of protein initially} \times 0.8}{\text{mL in collection tube}}$$

Biotinylating 1 mL of 0.5–3 mg/mL Antibody

2.1 Transfer 1 mL of a 0.5–3 mg/mL antibody solution to a 2 mL reaction tube containing a stir bar (Component B). Add 100 µL of a freshly prepared 1 M sodium bicarbonate solution.

2.2 Add 200 µL of distilled water to one vial of biotin-XX, sulfosuccinimidyl ester, sodium salt (Component A). Pipet the contents of the vial up and down to completely dissolve. Because this reactive form of biotin-XX rapidly hydrolyzes in water, you must prepare the aqueous solution immediately before use.

2.3 Refer to Table 3 for the amount of reactive biotin-XX solution that should be added to different concentrations of antibody solution to achieve approximately 3–8 biotin molecules per IgG.

If necessary, you may carry out the biotinylation reaction with 0.5–2 mL of antibody solution, but the amount of 1 M sodium bicarbonate and of biotin-XX solution must be reduced in proportion to the volume of antibody to maintain the appropriate concentrations of these reagents in the reaction mixture. The volume of reactive biotin-XX solution recommended in Table 3 for a given protein concentration (mg/mL) and protein solution volume (mL) should also be effective when biotinylating proteins other than antibodies, or immunoglobulin classes other than IgG.

Table 3. Amount of reactive biotin-XX solution to use with different concentrations of antibody (Ab) solution.

Concentration of Ab solution (mg/mL)	Volume of Ab solution (mL)	Amount of biotin-XX solution (µL)
0.5	1.0	9
1.0	1.0	12
1.5	1.0	16
2.0	1.0	22
2.5	1.0	26
3.0	1.0	30

2.4 While stirring, add the appropriate amount of reactive biotin-XX solution to the reaction tube containing the antibody and sodium bicarbonate. Mix thoroughly.

2.5 Stir the reaction mixture for 1–1.5 hours at room temperature.

2.6 Once the biotinylation reaction has proceeded for 1–1.5 hours, remove one piece of dialysis tubing (Component E) from the refrigerator and rinse it in distilled water. Tie a knot to one end of the dialysis tubing, squeeze out the excess distilled water, and transfer the entire reaction mixture to the tubing. Then, tie a knot in the other end of the dialysis tubing, leaving as little space between the knots as possible to prevent further dilution of the antibody. If handled correctly, the dialysis tubing will not break easily; however, the knot at each end of the tubing must be tight enough to ensure that the protein solution does not leak out during dialysis.

2.7 Hang the dialysis tubing in a 1 L beaker containing a stir bar filled with PBS or other desired buffer. Dialyze at 2–8°C for 24 hours with 3 to 4 changes of buffer. During the dialysis, the buffer should be gently stirring.

2.8 For most whole IgGs, the absorbance at 280 nm of a 1 mg/mL solution in a cuvette with a 1 cm pathlength is about 1.3–1.4. Therefore, the concentration (mg/mL) of the biotinylated antibody preparation can be determined by measuring the absorbance of the dialyzed sample at 280 nm and dividing this value by 1.3 or 1.4. Biotin does not absorb at 280 nm.

Product List Current prices may be obtained from our website or from our Customer Service Department.

Cat. no.	Product Name	Unit Size
F6347	FluoReporter® Mini-biotin-XX Protein Labeling Kit *5 labelings of 0.1–3 mg protein each*	1 kit

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