*Revised: 10–October–2001*

# *DSB-X™ Biotin Protein Labeling Kit (D-20655)*

**Quick Facts**

***Storage upon receipt:***
- 4°C
- DO NOT FREEZE

## *Introduction*

Molecular Probes' DSB-X™ Biotin Protein Labeling Kit provides a method for efficiently labeling small amounts of antibodies or other proteins with the unique DSB-X biotin ligand. DSB-X biotin is a derivative of desthiobiotin, a stable biotin precursor (Figure 1) that has the ability to bind biotin-binding proteins, such as streptavidin and avidin. Whereas harsh chaotropic agents and low pH (6.0 M guanidine HCl, pH 1.5) are required to dissociate the stable complexes formed between biotin and streptavidin or avidin,[1] DSB-X biotin can be readily displaced by applying an excess of D-biotin (B-1595, B-20656) or D-desthiobiotin (D-20657) at room temperature and neutral pH. As a result, DSB-X biotin conjugates can be reversibly bound to streptavidin or avidin. The subsequent removal of the biotin-binding protein allows for the reprobing of the DSB-X biotin conjugate, for example. Alternatively, streptavidin agarose can be used to temporarily immobilize the DSB-X biotin conjugate (e.g., an antibody) to allow for the capture of a target molecule (e.g., the antigen corresponding to the antibody). Once captured, the DSB-X biotin conjugate, along with the captured target molecule, can be released from the matrix for analysis.

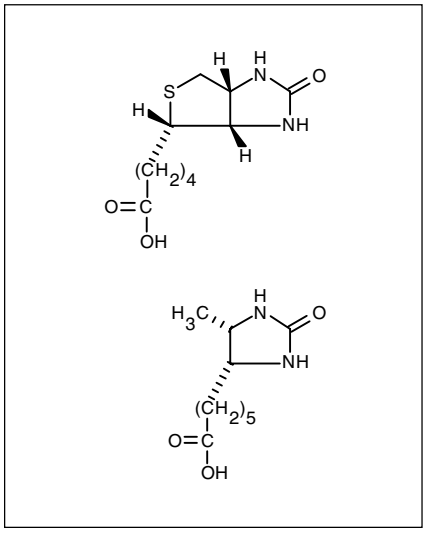

***Figure 1.*** *Comparison of the structures of D-biotin (top) to D-desthiobiotin (bottom).*

The DSB-X Biotin Protein Labeling Kit includes five vials of the amine-reactive DSB-X biotin succinimidyl ester. This reagent utilizes a seven-atom spacer to increase the ability of the DSB-X biotin moiety to bind in the deep biotin-binding pocket of streptavidin, avidin or other biotin-binding proteins.[2,3] Also included in the kit are dimethylsulfoxide (DMSO) for dissolving the DSB-X biotin succinimidyl ester, reaction tubes, purification resin for the spin columns, spin columns, collection tubes and dialysis tubing. The spin columns, when packed with purification resin, provide an extremely convenient method of purifying small volumes (e.g., 0.2 mL) of the labeled protein from any unreacted reagents. Alternatively, for larger protein volumes (e.g., 1.0 mL), excess reagents may be removed by dialysis, which avoids further dilution of the labeled protein. The DSB-X Biotin Protein Labeling Kit contains sufficient reagents for five reactions of 0.5–3 mg each.

## *Materials*

### *Kit Contents*

- **DSB-X™ biotin, succinimidyl ester** (Component A), 5 vials of 200 µg each
- **Dimethylsulfoxide (DMSO), anhydrous** (Component B), 500 µL
- **Reaction tubes** (Component C), five 2 mL tubes, each containing a stir bar
- **Purification resin** (Component D), 10 mL of sedimented resin with a size exclusion of ~30,000 MW
- **Spin columns** (Component E), five
- **Collection tubes** (Component F), five
- **Dialysis tubing** (Component G), five pieces, each with a size exclusion of ~12,000–14,000 MW

### *Storage and Handling*

Upon receipt, all kit reagents should be stored refrigerated at 4°C until required for use. DO NOT FREEZE THE PURIFICATION RESIN. When stored properly, the kit components should be stable for approximately six months.

### *Materials Required but Not Provided*

- **Phosphate-buffered saline (PBS)** — Dissolve 0.36 g of $NaH_2PO_4 \cdot H_2O$, 1.02 g of $Na_2HPO_4$ and 8.77 g of NaCl in 750 mL of deionized water ($dH_2O$), adjust the pH, if necessary, to 7.2 with 1 M NaOH or 1 M HCl and bring the volume to 1000 mL with $dH_2O$.
- **1 M sodium bicarbonate** — Dissolve 8.4 g of $NaHCO_3$ in 100 mL of $dH_2O$; the pH should be about 8.3–8.5.

## Protocol

This kit can be used to label virtually any protein, although the following protocol has been optimized for labeling IgG antibodies.

### Preparing the Antibody

The antibody must be purified from serum and other proteins before it is labeled with DSB-X biotin. If the purified antibody is at a concentration of 0.5–3 mg/mL in dilute buffer, such as 10–20 mM sodium phosphate buffer, then it may be used directly in the following protocols. However, if the purified antibody is in a buffer containing primary amines (e.g., Tris or glycine) or ammonium ions, then it must first be desalted either by using one of the spin columns provided (for a sample volume of 50–200 µL, see steps 1.6–1.12) or by dialyzing in PBS (for a sample volume of 0.5–2 mL, see steps 2.6–2.7). The presence of low concentrations of biocides, including sodium azide (≤3 mM) and thimerosal (≤1 mM), will not significantly affect the reaction.

### Small-Volume (0.2 mL) Labeling Reaction

The following protocol is for labeling a 0.2 mL volume of antibody at 0.5–3 mg/mL. For larger volumes of antibody, please refer to steps 2.1–2.8.

**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 C). Add 20 µL of a freshly prepared 1 M sodium bicarbonate solution.

**1.2** Add 40 µL of DMSO (Component B) to one vial of DSB-X biotin succinimidyl ester (Component A). Pipet up and down to completely dissolve the contents of the vial. To ensure the reactivity of the succinimidyl ester, the solution in DMSO should be made immediately before use.

**1.3** Use Table 1 to determine the amount of reactive DSB-X biotin solution to add for different concentrations of antibody solution. The reaction conditions are designed to result in 3–8 DSB-X biotin molecules covalently bound per antibody molecule. If necessary, the reaction may be carried out with smaller volumes (50–200 µL) of antibody solution, but the amount of 1 M sodium bicarbonate and of DSB-X biotin solution must be reduced in proportion to the volume of the antibody to maintain the appropriate concentrations of these reagents in the reaction mixture. The volume of reactive DSB-X biotin solution recommended in Table 1 for a given protein concentration (mg/mL) and protein solution volume (mL) should also be effective when labeling proteins other than antibodies, or immunoglobulin classes other than IgG.

***Table 1.*** *Amount of reactive DSB-X biotin solution to use with 0.2 mL of an antibody (Ab) solution at various concentrations.*

| Concentration of Ab solution (mg/mL) | Volume of Ab solution (mL) | Amount of DSB-X biotin solution to use (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 DSB-X biotin solution to the reaction tube containing the antibody and sodium bicarbonate, and mix thoroughly.

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

**1.6** Place a spin column (Component E) in a 13 × 100 mm glass tube.

**1.7** Stir the purification resin (Component D), then add 1.0 mL of the suspension into the column and allow the resin to settle.

**1.8** Continue to add more of the suspension until the bed volume is ~1.5 mL. Allow the column buffer to drain from the column by gravity. Initially, some pressure may be required to cause the first few drops of buffer to elute. Discard the column buffer, but not the collection tube.

**1.9** Place the spin column in one of the provided collection tubes (Component F) and centrifuge the column for 3 minutes at 1100 × g using a swinging bucket rotor. To convert revolutions per minute (rpm) into relative centrifugal force (g-force), either 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 radius, in centimeters, is measured from the center of the centrifuge spindle to the bottom of the rotor bucket. Discard the buffer, but save the collection tube. The spin column is now ready for purifying the conjugated antibody.

**1.10** Load the reaction mixture (~200 µL) onto the center of the spin column. Allow the solution to absorb into the gel bed. If any precipitation has occurred during the reaction, the sample must be centrifuged for 5 minutes in a microcentrifuge before loading; only the supernatant should be loaded onto the column.

**1.11** Place the spin column into the empty collection tube and centrifuge for 5 minutes at 1100 × g.

**1.12** After centrifugation, the collection tube will contain the labeled antibody in approximately 200 µL of PBS, pH 7.2, with 2 mM sodium azide; free DSB-X biotin will remain in the column.

**1.13** Typically, about 80–90% of the antibody in the reaction is recovered as an DSB-X biotin conjugate. Thus the concentration of the labeled antibody solution can be approximated using the following equation.

$$\text{mg/mL DSB-X biotin-labeled protein} = \frac{\text{initial mg of protein} \times 0.85}{\text{mL in collection tube}}$$

### Large-Volume (1 mL) Labeling Reaction

The following protocol is for labeling a 1.0 mL volume of antibody at 0.5–3 mg/mL. For smaller volumes of antibody, please refer to steps 1.1–1.13.

**2.1** Transfer 1.0 mL of a 0.5–3 mg/mL antibody solution to a reaction tube containing a stir bar (Component C). Add 100 µL of a freshly prepared 1 M sodium bicarbonate solution.

**2.2** Add 40 µL of DMSO (Component B) to one vial of DSB-X biotin succinimidyl ester (Component A). Pipet up and down to completely dissolve the contents of the vial. To ensure the reactivity of the succinimidyl ester, the DMSO solution should be made immediately before use.

**2.3** Use Table 2 to determine the volume of reactive DSB-X biotin solution that should be added to different concentrations of antibody solution. The reaction conditions are designed to result in 3–8 DSB-X biotin molecules covalently bound per antibody molecule. If necessary, the reaction may be carried out with different volumes (0.5–2.0 mL) of antibody solution, but the amount of 1 M sodium bicarbonate and of DSB-X biotin solution must be adjusted in proportion to the volume of the antibody to maintain the appropriate concentrations of these reagents in the reaction mixture. The volume of reactive DSB-X biotin solution recommended in Table 2 for a given protein concentration (mg/mL) and protein solution volume (mL) should also be effective when labeling proteins other than antibodies, or immunoglobulin classes other than IgG.

**2.4** While stirring, add the appropriate amount of reactive DSB-X biotin solution to the reaction tube containing the antibody and sodium bicarbonate and mix thoroughly.

***Table 2.*** *Amount of reactive DSB-X biotin solution to use with 1.0 mL of antibody (Ab) solution at various concentrations.*

| Concentration of Ab solution (mg/mL) | Volume of Ab solution (mL) | Amount of DSB-X biotin solution to use (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 |

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

**2.6** Remove one piece of dialysis tubing (Component G) from storage and rinse it in $dH_2O$. Tie a knot in one end of the dialysis tubing, squeeze out the excess $dH_2O$ 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 antibody 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 the reaction mixture at 2–8°C for 24 hours with 3–4 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 DSB-X biotin labeled 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. DSB-X biotin does not absorb significantly at 280 nm.

## Storage and Handling of Conjugates

Store the labeled antibody at 4°C. If the final concentration of purified antibody conjugate is less than 1 mg/mL, add bovine serum albumin (BSA) or other stabilizing protein to a concentration of 1–10 mg/mL. The conjugate should be stable at 4°C for several months. For long-term storage, divide the solution into single-use aliquots and freeze at -20°C. AVOID REPEATED FREEZING AND THAWING.

It is good practice to centrifuge conjugate solutions in a microcentrifuge before use; only the supernatant should then be used in the experiment. This step will remove any aggregates that may have formed during storage.

## References

**1.** Adv Protein Chem 29, 85 (1975); **2.** Biochemistry 21, 978 (1982); **3.** Biochemistry 23, 2554 (1984).

## *Product List* *Current prices may be obtained from our Web site or from our Customer Service Department.*

| Cat # | Product Name | Unit Size |
|---|---|---|
| B-1595 | D-biotin | 1 g |
| B-20656 | D-biotin *50 mM aqueous solution* | 10 mL |
| D-20657 | D-desthiobiotin *50 mM aqueous solution* | 10 mL |
| D-20655 | DSB-X™ Biotin Protein Labeling Kit *5 labelings* | 1 kit |

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Further information on Molecular Probes' products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Leiden, the Netherlands. All others should contact our Technical Assistance Department in Eugene, Oregon.

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