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Product Information

BiotinTag™ Micro Biotinylation Kit

Catalog Number **BTAG**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

The BiotinTag™ Micro Biotinylation Kit is designed for the small scale (1 mg) preparation of biotin-labeled polyclonal and monoclonal antibodies. These biotinylated antibodies may be used in ELISA, immunoblotting, and immunohistochemistry. The kit may also be used for conjugation of biotin to other proteins, such as peptide hormones or cytokines.

The BiotinTag Micro Biotinylation Kit provides reagents and a procedure for the small scale biotinylation (1 mg) of a protein (IgG), and Sephadex® G-50 micro-spin columns to purify the biotinylated protein.

Sufficient reagents are provided for at least 5 conjugations of biotinamidohexanoic acid 3-sulfo-N-hydroxysuccinimide ester (BAC-SulfoNHS) and a protein. The ExtrAvidin® Peroxidase conjugate is provided as a reporter molecule and may be used in avidin-biotin systems for the detection and assay of biotinylated proteins.

Note: For larger scale biotinylation of proteins (10 mg) use the ImmunoProbe™ Biotinylation Kit, Catalog Number BK101.

Purified proteins and antibodies are often obtained in small quantities for initial characterization and screening studies (i.e., biological activity, specificity) before larger scale preparation or production is undertaken. The availability of a small amount of protein may limit the number and variety of assays used in such screening studies. Proteins labeled with probes such as biotin, however, may help to overcome this limitation.

The avidin-biotin system provides a powerful tool for research and analysis due to the specific and high affinity ($K_a \sim 10^{15}\ M^{-1}$) interaction between avidin and biotin. Biotin-labeled antibodies and enzymes are used as powerful tools in a wide range of highly sensitive "nonradioactive" detection systems, where the amount of protein needed (nanograms) is extremely low. Such systems are used in standard immunological and immunocytochemical techniques,[1-5] and have been used in medical applications.[3] Also, biotinylated derivatives of peptide hormones, growth factors, cytokines, or other proteins are useful in various research applications involving affinity purification of receptors in combination with avidin affinity chromatography.[6,7] Biotinylation is often performed with N-hydroxysuccinimide (NHS) esters of biotin or biotinamidohexanoic acid (biotin-AC, BAC).[8] The use of the extended spacer arm greatly improves the interaction between avidin and the biotinylated macromolecule, thus, overcoming steric hindrance present at the biotin binding sites of avidin.[9]

Components

Biotinylation Reagent, BAC-SulfoNHS — 5 vials
(Catalog Number B4430)
Each vial contains 5 mg of biotinamidohexanoic acid 3-sulfo-N-hydroxysuccinide ester (BAC-SulfoNHS), MW = 556.8. BAC-SulfoNHS reacts with free amino groups of proteins to form stable amide bonds. For prolonged storage, keep desiccated at –20 °C.

Micro-spin Column G-50 — 5 columns
(Catalog Number M2287)
Micro-spin column, prepackaged with Sephadex G-50. Used for the separation of unreacted biotinylation reagent and buffer exchange. The column is preswollen in water containing 0.1% Kathon® CG/ICP as a preservative. **Do not freeze this reagent**.

0.1 M Sodium Phosphate Buffer, pH 7.2 — 1 vial
(Catalog Number P9693)
Used for dissolving and diluting the biotinylation reagent and as a suitable buffer for the biotinylation reaction.

0.01 M Phosphate Buffered Saline — 1 pack
(PBS), pH 7.4 (Catalog Number P3813)
This serves as equilibration buffer of the Sephadex G-50 column and for elution of the labeled protein from the column.

ExtrAvidin Peroxidase — 1 vial
(Catalog Number E2886)
Contains 0.2 ml of ExtrAvidin Peroxidase conjugate at 2.0–2.5 mg/ml. May be used as a reporter molecule in conjunction with the biotinylated protein in the avidin/biotin system. Supplied with 0.01% thimerosal.
Do not freeze this reagent.

Reagents and Equipment Required but Not Provided

- Protein for biotinylation
- UV/Visible spectrophotometer
- Variable speed microcentrifuge or low speed centrifuge (e.g., clinical centrifuge)
- Screw cap microcentrifuge tubes or flip-top Eppendorf® tubes (1.0–1.5 ml)
- 96 well plate (optional, for protein microconcentration determination)
- ELISA reader (optional, as above)
- Protein assay kit (optional, as above, Bicinchoninic acid [Catalog Number BCA1] or equivalent).
- DMSO (Catalog Number D5879)

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

0.1 M Sodium Phosphate Buffer, pH 7.2 - Reconstitute vial (Catalog Number P9693) with 6 ml of water.

0.01 M Phosphate Buffered Saline (PBS) - Add contents of Phosphate Buffered Saline, pH 7.4, pouch (Catalog Number P3813) to a suitable container. Add 800 ml of water and mix. Bring the final volume to 1 liter with water to give 0.01 M phosphate buffer, 0.138 M NaCl, and 2.7 mM KCl, pH 7.4.

Storage/Stability

Store the kit at 2–8 °C.

Procedure

The BiotinTag Kit is specially designed for the small scale labeling of antibodies (1 mg) using biotinamido-hexanoic acid 3-sulfo-N-hydroxysuccinimide ester (BAC-SulfoNHS) as the labeling reagent. This derivative is soluble in water and biotinylation proceeds at near physiological pH values. The reagent is particularly useful when mild reaction conditions are required for the biotinylation of sensitive biomolecules such as antibodies, enzymes, and cell surface proteins.[4,5] Following the labeling reaction, the biotinylated protein is separated from unreacted or hydrolyzed reagent by a fast gel-filtration step using G-50 micro-spin columns. The biotinylated protein may be used in various systems such as ELISA, immuno-histochemistry, immunoblotting, and dot-blot immunoassays.

Protein Biotinylation

The following procedure describes the preparation of biotin-labeled IgG. The molar ratio in the reaction mixture is ~13:1 of biotinylation reagent (BAC-SulfoNHS, MW = 556.8) to IgG (MW = 150,000). In most cases, this ratio results in 4–8 moles of biotin per mole of protein, respectively. This procedure can be modified if a protein with a different molecular mass, or a different amount of IgG or BAC-SulfoNHS/Protein ratio is used in the labeling reaction.
Notes: The level of biotin labeling may vary from one protein to another, partly due to different number of lysine residues available for modification.

The biotin/protein molar ratio of the biotinylated protein may be determined by the HABA-avidin assay.[10] However, this assay requires a larger amount of protein (1 mg). Refer to the ImmunoProbe Biotinylation Kit, Catalog Number BK101 for the large scale biotinylation of proteins (10 mg) and the application of the HABA-avidin assay.

1. Prepare 0.1 ml of IgG solution at 10 mg/ml in 0.1 M Sodium Phosphate Buffer, pH 7.2 (Catalog Number P9693). The A_{280} of a 1.0 mg/ml IgG solution equals 1.4, using a 1.0 cm pathlength.
   Note: Protein solutions should not be prepared in buffers containing amines such as Tris or glycine, or sodium azide since they inhibit the labeling reaction. If the buffer contains amines or sodium azide, dialyze against 0.1 M sodium phosphate, pH 7.2, or against PBS, pH 7.4.

2. Dissolve the contents of one vial of Biotinylation Reagent, BAC-SulfoNHS (Catalog Number B4430) with 30 µl of DMSO and then add 0.1 M sodium phosphate buffer, pH 7.2, to a final volume of 1 ml. Vortex well. The resulting concentration of the BAC-SulfoNHS solution is 5 mg/ml.
   Note: The solution of BAC-SulfoNHS should be prepared fresh, just before labeling as it is not stable in aqueous solutions. Biotinylation with BAC-SulfoNHS can be carried out in 100% aqueous solutions. However, greater solubility can be achieved when the reagent is first dissolved in a small amount of DMSO.
3. Immediately add 10 µl (molar ratio 13:1) of the BAC-SulfoNHS solution to the IgG solution with gentle stirring.
4. Incubate with gentle stirring for 30 minutes at room temperature or 2 hours at 2–8 °C.

Isolation of Labeled Protein

1. Use the reconstituted 0.01 M Phosphate Buffered Saline (PBS) as the equilibration buffer for the micro-spin G-50 column and for elution of the labeled protein from the column. For prolonged storage, store the buffer at 2–8 °C for up to 3 months.
2. Resuspend the resin in the column by vortexing.
3. Loosen the cap one-fourth turn and snap off the bottom closure.
4. Place the column in a 1.0–1.5 ml screw cap microcentrifuge tube for support. Alternatively, cut the cap from a flip-top Eppendorf tube and use it as a support.
5. Pre-spin the column for 1 minute at 700 × *g*.
   Note: Use an Eppendorf variable speed centrifuge Model 5415 set at 3,000 rpm (~700 × *g*). Alternatively, if you do not have a microcentrifuge use a low speed centrifuge set at 700 × *g*. Place the column in a 1.5 ml microcentrifuge tube for support and set the support tube in a 100 mm × 13 mm test tube. Place the entire system in a low speed centrifuge and proceed as previously described.
6. Equilibrate the column in PBS, pH 7.4. Apply 0.2 ml of PBS, pH 7.4, to the column. Spin the column for 1 minute at 700 × *g*. Repeat this step once.
7. If the column is not immediately used, wash the column as in step 6 with PBS, pH 7.4. Close column with the top cap, seal the bottom with Parafilm® and store the column at 2–8 °C.
8. Label each of four 1–1.5 ml tubes as 1, 2, 3, and 4.
9. Place the column in tube 1 and apply the biotinylation reaction mixture to the top-center of resin, being careful not to disturb the bed.
10. Spin the column for 2 minutes at 700 × *g*. The purified sample is collected at the bottom of the support tube (fraction 1).
11. Elute the column with PBS, pH 7.4. Place the column into tube 2 and apply 0.2 ml of PBS, pH 7.4, to the column. Spin the column for 1 minute at 700 × *g*. Repeat this step twice more in tubes 3 and 4. A total of four fractions are obtained.
    Note: Discard the micro-spin column after its use. Do not attempt to regenerate it.
12. Determine protein concentration (optional) in each of the collected fractions 1–4 by protein microassay (e.g., Bicinchoninic Acid Kit for Protein Determination, Catalog Number BCA1).
    Notes: Determination of protein concentration by measuring absorption at 280 nm is not recommended, since it does not give exact results (deviations of ±20%). The micro-spin columns contain a preservative that absorbs at 280 nm. Traces of this preservative elute from the column along with the protein and interfere with the 280 nm measurement. The preservative does not interfere in immunological assays using the biotinylated antibody. Protein determination by a colorimetric assay is optional. If step 12 is omitted, proceed directly to step 13.
13. Pool fractions containing the protein. When collecting fractions of 0.2 ml, the labeled protein is present in fractions 1, 2, and 3. If needed, fraction 4 can be pooled. If the protein concentration of this fraction is low, it can dilute the sample. Do not pool fractions after fraction 4.
14. The biotinylated protein is now ready to use. Applications include ELISA, immunoblotting, dot blot immunoassy, or immunohistochemistry.

References

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