Biotinylation Kit

Catalog # IM30

Intended Use

This kit is intended for simple and reliable biotinylation of proteins through covalent binding to primary amine groups on the protein of interest. The kit also contains a prepacked, factory calibrated Sephadex^{TM} G25 column for rapid purification of the biotinylated protein. Biotin:Protein conjugates can provide useful labels for sensitive and specific analytical techniques using streptavidin and immunologcial binding. The kit is for research use only and is not intended for diagnostic use in humans or animals.

Summary and Explanation

Owing to the high avidity and specificity of biotin for its binding proteins avidin and streptavidin, the labeling of proteins with biotin or biotin derivatives can create powerful analytical tools. Many immunoassay methods such as ELISA can be made to yield amplified signal and sensitivity by exploiting binding of biotinylated protein target molecules to streptavidin and streptavidin:enzyme conjugates. Streptavidin or avidin immobilized on to solid supports such as chromatography media or microtiter plate wells are reliable and specific ways to purify or immobilize biotinylated proteins like antigens or antibodies.

Biotin can be covalently conjugated to protein by a number of methods. Typically these methods employ the use of commercially available activated biotinylation reagents capable of forming covalent bonds with various functional groups such as primary amines, sulfhydryl, carbohydrate, or carboxyl groups. Because most proteins will have some primary amine groups this kit utilizes an N-hydroxysuccinimide (NHS) ester of biotin capable of forming covalent bounds to primary amine groups. This kit provides all the reagents necessary for simple and reproducible target protein biotinylation as well as a rapid method to purify the biotinylated protein from excess unconjugated biotin.

Principle of the Procedure

The protein of interest must first be placed into a compatible buffer. Buffer salts containing amine groups such as glycine or tris buffers should not be used. Dialysis or other methods of buffer salt exchange can be employed to remove competing materials from the initial protein sample. Phosphate or carbonate buffers in the range of pH 6-9 can be used to efficiently biotinylate most proteins using the reagent provided in this kit. Because most proteins will have multiple primary amine groups, the desirable degree of biotinylation of those groups should be determined. Unless a molar excess of biotin is added to the protein, biotinylation of amine groups may proceed in ordered or random ways. Over biotinylation of a protein may result in some interference of its biological or immunological activity and thus the optimal ratio of biotin to protein may need to be experimentally optimized. If a particular amine group is critical to the function of that protein or its binding to other proteins and biotinlyation with the N-hydroxsuccinimide ester can be shown to interfere, you should consider the use of other biotinylation reagents that react with non-amine groups.

The biotinylation reaction itself proceeds spontaneously at the recommended buffer conditions and will generally be complete within 1 to 2 hours. At the end of the biotinylation reaction time, the biotinylated protein is purified over the prepacked, factory calibrated Sepahdex^{TM} G-25 column supplied in the kit to remove unconjugated biotin from your biotinylated protein.

Reagents & Materials Provided

Component	Product #
Ready-to-Use **Biotin-NHS** in solvent Biotin-NHS in Dimethylformamide (DMF) solvent 1mL. **Store at –10 to -30°C upon receipt.**	**IM31**
Sephadex^{TM} G25 Column 1 reuseable prepacked column	**IM32**
Column Elution Buffer Phosphate buffered saline, pH 7.0, 1x100mL	**IM33**

Storage & Stability

For long term storage (>1month) the Biotin-NHS reagent (Product #IM31) should be stored at –10°C to -30°C. Otherwise store all reagents at 2°C to 8°C.

Limitations of the Procedure

- Users of this kit are strongly encouraged to contact our technical services department for questions or advice on how best to approach biotinylation of your protein.
- The Biotin-NHS reagent (#IM31) is subject to hydrolysis by water and will loose coupling reactivity rapidly if exposed to aqueous solutions. Do not introduce water into the vial and avoid prolonged exposure to air to avoid adsorption of water vapor by the hydroscopic DMF solvent. **Tightly recap vial immediately after removing required volume and return to freezer storage!**

- If a particular amine group is critical to the function of your protein or to its binding to other proteins and biotinyation with the N-hydroxsuccinimide ester in this kit can be shown to interfere, you should consider use of other biotinylation reagents that react with non-amine groups.

Materials & Equipment Required But Not Provided

Your protein to be biotinylated in a compatible (non-amine containing) buffer.
Adjustable Pipettors – capable of accurately dispensing in the microliter ranges.

Precautions

For research use only. Not for clinical or diagnostic use in human or animals.

Biotin-NHS reagent is in Dimethylformamide solvent. Do not breath and avoid contact with skin and eyes.

At the concentrations and volumes used in this kit none of the other reagents are believe to be harmful.

This kit should only be used by qualified technicians.

Preparation of Reagents

The Sephadex™ G-25 column should be equilibrated into the Column Elution Buffer (IM33) prior to purifying your biotinlylated protein. Remove the top cap from the column and using a sharp knife or razor blade cut off the bottom tip of the column to allow the resident buffer to flow out of the column. Add approximately 20mL of Column Elution Buffer to equilibrate it adequately.

Your protein to be biotinylated needs to be in an amine-free buffer system, such as phosphate or carbonate buffered saline. Avoid buffers containing Glycine or Tris as these reagents will consume the Biotin-NHS reagent and thus reduce the biotinylation of your protein. Optimal pH is 7.0 - 7.5 but successful biotinylations can occur at pHs ranging from 6 to 9. We suggest a protein concentration of 1 to 2 mg/mL but good biotinylation can occur outside this range.

Biotinylation Procedure

The following procedure is offered as an example only and represents a typical biotinylation of an antibody without compromising its immunological activity. The correct molar ratio of biotin to protein needs to be experimentally determined for each protein. In general a good starting molar ratio of biotin to protein is 20:1 but users of this kit may consider varying the ratio from 2 to 100 to achieve optimal results.

Example: **Biotinylation Procedure for an Antibody Protein**

1. Buffer exchange the antibody into a 0.05 M Phosphate pH 7.0 with 0.15 M NaCl (PBS) buffer to remove any interfering materials in the sample. Dilute the antibody to 1 mg/mL in PBS Buffer.
2. Add 30μL of the Biotin-NHS reagent (IM31) per mg of antibody to the vial containing the antibody.
3. Cap and allow to react at 4°C for 2 hours.
4. Purify on Sephadex™ G25 column as described in the purification section below.

Purification Procedure

1. Equilibrate the Sephadex™ G25 column into the Column Elution Buffer as described in the section on **Preparation of Reagents.**
2. The void volume of this column is approximately 2.5 mL. Therefore, if your sample is in less than 2.5 mL you may dilute it to 2.5 mL in Column Elution Buffer. Sample volumes greater than 2.5 mL must be put through the column in 2.5 mL aliquots, since the column is re-useable after sufficient washing.
3. Apply 2.5 mL of sample to the top of the column and allow to flow into the gel bed. (Flow will automatically stop due to liquid surface tension as soon as the entire liquid sample has entered the bed.) Discard 2.5mL of eluate.
4. Place a collection tube under the column and add 3.5mL of Column Elution Buffer. Greater than 98% of your biotinylated protein will be in this 3.5mL elution while more than 99% of the unconjugated biotin will remain on the column.
5. To re-use the column for future purifications wash the column with at least 20mL of column buffer to completely remove the low molecular weight unconjugated biotin.

Ordering Information/ Customer Service

To place an order or to obtain additional product information contact *Cygnus Technologies* Customer Support:
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