



Biotin (Long Arm) NHS - Water Soluble

Cat. No. SP-1210

Although a variety of methods have been used to biotin label proteins, the following procedure is one of the simplest and most reliable. Some modifications may be required for specific proteins.

Reagent preparation: Biotin (Long Arm) N hydroxysuccinimide ester-Water Soluble (BNHSws, Cat. No. SP-1210) reacts with amino groups on proteins. This reagent is prepared by dissolving BNHSws in deionized water at a concentration of 25-50 mg/ml. It is recommended to make up slightly more reagent than is needed so that accurate amounts can be aliquoted. Only the required amount of solid reagent should be weighed out and the remainder stored desiccated at 4 °C. It may be necessary to warm the solution slightly to completely dissolve the BNHSws. This reagent should be prepared just prior to use.

Procedure: Dissolve the protein to be biotinylated in 100 mM HEPES or sodium bicarbonate buffer, pH 8.5, at a concentration between 2 mg/ml and 10 mg/ml. Add an aliquot of the biotinylating reagent equal to 1/10 the weight of the protein to be labeled (e.g., for labeling 10 mg of protein in 1 ml, add 40 µl of a 25 mg/ml solution of the BNHSws reagent). With occasional stirring, incubate at room temperature for 2 hours. To stop the reaction, add 10 mg of glycine, or 5 µl of ethanolamine. Dialyze the biotinylated protein against 3 changes of 2 liters of buffer or remove unreacted biotin by gel filtration. The percent biotinylation can be adjusted by increasing or decreasing the amount of BNHSws.

Assay for Percent Biotinylation

Principle: Generally most proteins are biotinylated to about 95% with the above protocol as measured by the following assay method. After removal of any free biotin following the biotinylation procedure, an aliquot is taken and passed through a small column containing Agarose Avidin D (Cat. No. A-2010). By measuring the amount of protein passing through the column, the percentage of the biotinylated protein capable of binding avidin (i.e. percent biotinylation) can be determined. This is generally the best criterion to assess the degree of biotinylation of a protein.

Procedure: Place a small plug of fine glass wool in a pasteur pipet. Add 2 ml of a 1:1 slurry of Agarose Avidin D to this column. Wash the gel with 4-5 column volumes of phosphate buffered saline (PBS) or until the OD₂₈₀ of the effluent equals that of the PBS. Remove an aliquot* from the dialyzed biotinylated protein and add it to the top of the Agarose Avidin D column. Collect the displaced volume in a test tube and wash the column by gravity with 1.0 ml of PBS. Collect this effluent in the same test tube (Solution A). In a second test tube, add the same size aliquot from the biotinylated protein solution to 1.0 ml of PBS (Solution B). Measure the OD₂₈₀ of Solution A and Solution B.

The percent biotinylation is considered to be the percentage of the protein which binds to the Agarose Avidin D. This value is derived using the following equation:

$$\% \text{ Biotinylation} = \frac{\text{OD}_{280} (\text{Solution B}) - \text{OD}_{280} (\text{Solution A})}{\text{OD}_{280} (\text{Solution B})} \times 100$$

For example, if 50 µl of biotinylated protein diluted with 1.0 ml of buffer results in an OD₂₈₀ of 0.25, and the OD₂₈₀ of the effluent from applying 50 µl of sample to the Agarose Avidin D column and washing it with 1.0 ml of buffer is 0.025, the protein is considered to be 90% biotinylated. If the OD₂₈₀ of the effluent is 0.0025, the protein would be 99% biotinylated, etc.

* The size of the aliquot should be such that when added to 1.0 ml of PBS, the OD₂₈₀ is between 0.15 and 0.30. This provides sufficient protein for accurate measurement of percent biotinylation but generally is not enough to saturate the column.

Notes:

1. The Agarose Avidin D has a defined molar binding capacity for biotinylated proteins. Agarose Avidin D will bind less by weight of a small molecular weight biotinylated protein than a larger one.
2. If the amount of protein to be biotinylated is small, it is suggested that another protein can be biotinylated in parallel and assayed for percent biotinylation, ensuring that all reagents are functioning properly.