Biotinylation of Antibodies

Overview:

Biotin is used in two-step detection systems in concert with conjugated avidin. Biotin is typically conjugated to proteins via primary amines (i.e., lysines). Usually, between 3 and 6 biotin molecules are conjugated to each antibody.

Refer to notes about the following procedures used by this protocol:
Column chromatography
Reagent storage

Contents:

Conjugation protocol.

The entire conjugation can be performed in about a half-day. In addition to the materials listed below, you will need to have a solution of your antibody at a concentration (optimally) of at least 2 mg/ml. The extent of biotin conjugation to the antibody may depend on the concentration of antibody in solution; for consistent conjugations, use a consistent concentration. You should be familiar with how to use a desalting column and how to take absorbance spectra.

The reactive biotin molecule is unstable. Once the biotin is solubilized, it should be used immediately.

When first conjugating an antibody, a range of biotin to antibody concentrations should be compared. The protocol suggests 80 µg per mg of antibody; for a first-time titration of biotin, try a range of 10 to 400 µg biotin per mg of antibody (for instance, 10, 40, 80, 160, 320 µg per mg). Compare each conjugate by staining (you should perform a titration of antibody on cells for each reagent to determine the optimal staining concentration). Select the conjugate with the brightest "positive" cells which still has low background on "negative" cells.

If this procedure fails, you may try either using "long-arm" biotin (NHS-LC biotin II) or biotinylating carbohydrates (Biotin-LC Hydrazide).

I. Preparation of antibody

Note: it is critical that sodium azide be completely removed from any antibody.

Dialyze or exchange over a column the antibody in "B Reaction Buffer".

Measure the antibody concentration after buffer equilibration. (For IgG, 1 mg/ml has an A(280) of 1.4). If the

antibody concentration is less than 1 mg/ml, the conjugation will probably be sub-optimal. If necessary, dilute the antibody to a concentration of 4 mg/ml.

II. Covalent conjugation

Biotin is covalently coupled to primary amines (lysines) of the immunoglobulin.

Dissolve 10 mgs of biotin in 1 ml anhydrous DMSO immediately before use.

Add biotin to give a ratio of 80 µg per mg of antibody; mix immediately. (See notes above about using different molar rations of biotin to antibody).

Wrap the tube in foil; incubate and rotate at room temperature for 4 hours.

Remove the unreacted biotin and exchange the antibody into "Storage Buffer" by gel filtration or dialysis.

Materials, Chemicals, and Buffers

Materials:

For column separations, we often use one of two types of pre-poured columns:

For 1.25ml to 2.5ml sample volumes: PD-10 (Sephadex G-25M).

For <0.5 ml sample volumes: NAP5 columns (Sephadex G-25 DNA grade).

Chemicals:

DMSO - anyhydrous dimethyl sulfoxide.
Note: keep the DMSO absolutely dry at all times. *We keep the bottle in a dessicator. Pour out an amount of DMSO sufficient for your need and then pipette that; don't pipetter directly into the bottle.*

NaHCO3 - sodium bicarbonate, mw 84.01

NaCO3 - sodium carbonate, mw 106

NaCl - Sodium Chloride, mw 58.44

TRIZMA pre-Set crystals 8.0 - Combination of Tris base and TrisHCl, average mw 141.8

NaN3 – Sodium Azide, mw 65

Buffers:

"B Reaction Buffer"
100 mM carbonate, pH 8.4

To make 1 Liter:
84g NaHCO3
pH to 8.4
Note: sodium azide cannot be added to this buffer

"Storage Buffer"
10 mM Tris, 150 mM NaCl, 0.1% NaN3, pH 8.2

To make 1 Liter:
1.42g TRIZMA 8.0
8.77g NaCl
1g NaN3
pH to 8.2

See hints on storing buffers.

References and credits:
This protocol is based on an original protocol devised by Aaron Kantor.