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USE OF BIOTIN-ACTIVATED ESTERS FOR PROTEIN CONJUGATION

Principles:

Biotin is a water soluble molecule which is fairly polar, and can be conjugated to proteins with relatively little disruption of their structure (unlike nitrophenyl and related compounds). The availability of avidin, an egg white glycoprotein (M.W. 66,000) which binds extremely tightly to biotin, allows a convenient second step reagent.

Biotin itself must be derivatized before it can be coupled to proteins. The most convenient compounds are the succinimide or nitrophenyl esters. The succinimide ester is somewhat more "active" (i.e., the coupling efficiency is higher), but the nitrophenyl ester is perfectly satisfactory if more is used.

Both compounds are readily hydrolyzed. They must be kept dry, and should be weighed out just prior to use. Because of their susceptibility to hydrolysis, the efficiency of coupling to protein will depend on the protein concentration. At low protein concentrations, most of the biotin ester will be hydrolyzed. At higher concentrations of protein, most of the biotin will couple to protein. The conditions described here have been worked out to give optimal conjugation of IgG. If the protein concentration is altered, the optimal amount of biotin ester will be different. Unless there is compelling reason to do so, I suggest that you do not deviate from the protocol. Under the conditions used (protein at 1.0 mg/ml), about 10% of the biotin succinimide ester will couple to protein.

Use of the nitrophenyl ester is similar. Both esters couple to free amino groups at alkaline pH. However, the optimal amount of the nitrophenyl ester will be X2-X4 higher. Note that during coupling, p-nitrophenol (yellow) will be released.

There is no easy way to assess the conjugation ratio of biotinylated proteins.

Suppliers:

Biotinyl-p-nitrophenyl ester. Calbiochem #203125. $25/250 mg (Als_Sigma Chemical) I

Biotin succinimide ester. Biosearch, 3095V Kerner Blvd, San Rafael, CA, 94901 (415-457-8515) $25/100 mg (not yet in catalog).

Biotin and avidin. Sigma Chemical Co., P.O. Box 14508, St. Louis, MO, 63178

Rhodamine-avidin (Cat. #A-2002) and fluorescein-avidin (Cat. #A-2001) $45/5 mg. Vector Labs, 1479 Rollins Rd., Burlingame_ CA, 94010 (415-344-6161) Also available from E. Y. Laboratories, Inc., P. O. Box 1787, San Mateo, CA 94401 (415-342-3296). Agents in Palo Alto: M/S Scientific Assoc., 258 Solana Dr., Los Altos, CA, 94022 (415-941-2589).

Biotin-Activated Esters, cont.

Practical Procedure:

1. Antibody should be fairly pure IgG. Ammonium sulfate fractions are OK, but be certain to dialyze extensively to remove the ammonium sulfate.

2. Dialyze protein against 0.1 M NaHC03 (no azide or other preservative). pH should be 8.2-8.6 approx. (it is not usually necessary to measure pH).

3. Adjust protein to 1.0 mg/ml.

4. Weigh out biotin succinimide ester (warm bottle to room temperature before opening, to prevent condensation).

5. Dissolve in DMSO (immediately before use) to 1.0 mg/ml.

6. Add 120 µl per ml protein. Mix immediately.

7. Leave at room temperature for 4 hours.

8. Dialyze against PBS with azide overnight.

NOTE:

For optimal staining, it may help to titrate the ratio of biotin:protein (try 60 vl/ml :240 vl/ml). Too little gives weak staining. Too much gives nonspecific staining. 120 vl/ml protein is usually optimal.

References:

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4. Heitzmann, H. and F. M. Richards. 1974. Use of the avidin-biotin complex for specific staining of biological membranes in electron microscopy. Proc. Natl. Acad. Sci. 71: 3537-3541.
5. Becker, J. M., t1. Wilchek and E. Katchalski. 1974. Irreversible inhibition of biotin transport in yeast by biotinyl-p-nitrophenyl ester. PNAS 68: 2604-2607.
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7. Pohlit, H. M., W. Haas and H. von Boehmer. 1979. Haptenation of viable biological carriers. In: Immunological Methods, (eds) I. Lefkovits and B. Pernis, Academic Press, pp. 181-194. (A useful account of succinimide esters)