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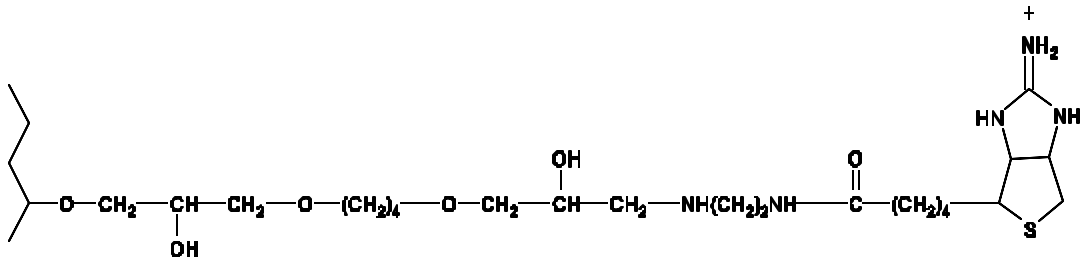
ProductInformation

2-IMINOBIOTIN AGAROSE
Sigma Prod. No. I4507

The structure of the linker arm with 2-iminobiotin in protonated form is given below.[1]



DESCRIPTION:

Matrix: 4% beaded agarose
Activation: epoxy, with attachment through the carboxyl group
Spacer: 16 atoms
Binding capacity: minimum 4 mg avidin per ml
Form: suspension in 0.5 M NaCl, 0.01 M sodium phosphate, pH 6.8, containing 0.02% sodium azide

METHOD OF PREPARATION/STRUCTURE:

A 4% beaded agarose is activated with 1,4-butanediol diglycidyl ether. The epoxy agarose is then treated with ethylenediamine, coupled with 2-iminobiotin, then blocked with acetic acid N-hydroxysuccinimide ester. [1] Other methods of attachment have been reported.[2,3,4,5]

GENERAL REMARKS:

Much is written in the literature about the interactions of avidin-biotin, streptavidin-biotin and monomeric avidin interacting with biotin. The binding of biotin to avidin is very strong, with a K_D approximately 10^{-15}; streptavidin is reported to have a similar K_D value. However, iminobiotin has a much lower dissociation constant which is pH-dependent. The pKa of iminobiotin is in the range of 11-12, with a K_D = 3.5 x 10^{-11} in base, but at pH 3-4, the K_D of the protonated iminobiotin <10^{-3} .[2]

$$>C{=}NH \;:\; >C{=}NH_2^+$$
high pH low pH

GENERAL REMARKS: (continued)

The conditions for separating either avidin-biotin or streptavidin-biotin complexes are extremely harsh, requiring the use of 6 M guanidine salts and pH # 1.5. Under these conditions, most proteins denature totally and cannot be recovered.[3,4] Avidin and streptavidin are stable to pH extremes, but proteins conjugated to them are less stable. Using iminobiotin labels or iminobiotin-agarose, lowering the pH to about 4 with acetate buffer is often enough to protonate the ligand, releasing the streptavidin-labeled protein.

USAGE / EQUILIBRATION:

The resin must be well-washed before use to remove the storage buffer; it is best to use a basic buffer such as 50 mM ammonium carbonate or sodium borate at pH 11, containing 0.5 M NaCl, in several 50-mL aliquots. The beads can be washed on a sintered glass filter using gentle vacuum suction, but care must be taken that the beads not be crushed or completely dried during filtration.

A protein sample applied to a column of the resin should be equilibrated in buffer pH 11 containing 0.5 M NaCl. Once binding has occurred, the resin should be washed with the same pH 11 buffer until the absorbance of effluent at 280 nm reaches blank values.

Elution with 50 mM ammonium or sodium acetate pH 4.0 will release avidin or streptavidin, detectable by absorbance measurements at 280 nm.[4,5]

By analogy with immobilized avidin and elution of 2-iminobiotin or its conjugates, it is likely that the use of 0.1% (w/v) Triton X-100, Tween 80, sodium deoxycholate will NOT interfere with the affinity isolation. The use of sodium dodecyl sulfate (SDS) completely destroyed all binding.[6]

The horseradish peroxidase and alkaline phosphatase conjugates of avidin/streptavidin can also be purified by chromatography on a 2-iminobiotin column. The column is equilibrated at pH 10 rather than at pH 11, and specific elution is achieved with pH 6 buffer.[2]

In the analogous system previously mentioned, specific elution was achieved using pH 4.0 buffer or by using 50 mM Tris-HCl, pH 6.8 containing 1 mM biotin. This had the advantage of requiring a less acidic buffer, but the disadvantage that the biotin selectively binds (essentially permanently) to the avidin-agarose, displacing the iminobiotin analog.

The affinity resin can be regenerated by washing with pH 11 buffer, then stored in a buffer at pH 7 containing 0.5 M NaCl and suitable preservative at 0-8°C.

REFERENCES:

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2. Methods in Enzymology, 184, 167 (1990). Iminobiotin affinity columns.
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6. Orr, G.A., J. Biol. Chem., 256, 761-766 (1981). Using avidin-agarose for selective retrieval of iminobiotin-labeled molecules.

ADDITIONAL READING:

Green, N. M., BIOCHEM. J., 101, 774 (1966).