ProductInformation

L-CYSTEINE - AGAROSE
Sigma Prod. No. C7896

CAS NO.: N/A

PHYSICAL DESCRIPTION:
Appearance: White suspension (2 mL suspension = 1 mL gel)
Matrix: 4% beaded agarose activated by cyanogen bromide
Attachment: Amino group
Spacer: 1 atom
Ligand immobilized: 0.5-1.0 μmole/mL gel
Form: suspension in 0.5 M NaCl, 0.01 M citrate buffer, pH 4.5, containing 0.01% thimerosal as bacteriostat

STORAGE / STABILITY AS SOLD:

This agarose resin should be stored at 2-8°C. Freezing the suspension may damage bead structure, affecting flow rate and possibly binding properties. When stored sealed, this product has retained full activity of its cysteine sites (by DTNB titration-see below) for at least two years.

USAGE:

The resin should be washed well in several aliquots of equilibration buffer (Tris-HCl, for example), using 30-50 mL buffer per mL resin for each wash. The beads may be packed into a small column; gravity flow will determine the flow rate.

Several references speak in very general terms about the use of immobilized cysteine and similar resins, but no specific protocols have been reported. Some suggestions have been summarized below.

In order for the cysteine -SH to form disulfide bridges with a protein, both the resin and protein the thiol groups of must first be in reduced form. Under oxidizing conditions, thiol groups on the target protein should link to the immobilized thiol groups and remain bound to the agarose. The bound proteins can then be recovered by adding a reducing agent to the equilibration buffer. L-cysteine at 5-20 mM is fairly mild; its use would minimize unwanted reduction of intramolecular disulfide bridges. 2-Mercaptoethanol (2-ME) is also useful, since it is easily removed. Stronger reducing agents such as reduced glutathione and dithiothreitol (DDT) can be used as well.

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USAGE:

The resin cannot be routinely reassayed using amino acid analysis. The cysteine content of the resin can be determined using the DTNB procedure:[1]

Filter the gel and wash it several times with distilled water. Take 100-300 mg of the damp cake and suspend it in 8 mL 0.1 M Tris buffer, pH 8.0. Add 0.25 mL DTNB (5',5'-dithio-bis(2-nitrobenzoic acid), D8130) solution. Bring the volume to 10 mL with 0.1 M Tris, pH 8.0 (note the exact volume). Shake 10 min. Filter or centrifuge to remove the gel. Read the absorbance of the supernatant at 412 nm.

To calculate μmol cys/g gel:

(μmol cys/ΔOD at 412 nm) X (10 mL/wt of gel in g) X 13.6 X 1.1 = μmol cys/g gel.

13.6 is the E^{mM} of DTNB, and 1.1 is a conversion factor for weight in g of 1 mL of packed gel.

REFERENCES:

1. Sigma production department
2. Brocklehurst, K. et al., *Methods in Enzymology*, 34B, 531-544 (1974).
3. Hermanson, G.T., Mallia, A.K. and Smith, P.K., *Immobilized Affinity Ligand Techniques* (Academic Press, Inc., 1992), p. 274-277, 382-384.

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