3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

Adenosine 2',5'-diphosphate-Agarose

Product Number **A 3515**
Storage Temperature -0 °C

Product Description

Adenosine 2', 5' bis-phosphate is an NADP analog which, when coupled with the 8-atom spacer arm, is sterically acceptable to most NADP-dependent enzymes. For approximately 2 μmoles of ligand immobilized per ml of gel, the binding capacity for glucose 6-phosphate dehydrogenase (G6PDH) is approximately 0.4 mg per ml in a Tris buffer, pH 7.6, with EDTA and 2-mercaptoethanol.

This resin can also be used for the purification of guanylyl cyclase-activating factor synthase.[1] It will also bind $NADP^+$ dependent dehydrogenases and other $NADP^+$ binding enzymes. In general, elution can be obtained by using competing cofactors or gradient elution by ionic strength, pH, or temperature. Another usage for this resin is the initial purification step for separation of thioredoxin from thioredoxin reductase and glutathione reductase.[2]

This resin has also been used for the purification of glutathione reductase from porcine erythrocytes[3] and calf liver.[4] In the calf liver preparation, the dialyzed material was applied to a column equilibrated with 50 mM potassium phosphate, pH 7.5. After sample application, the column was washed with 0.4 M potassium phosphate, pH 7.5, until no additional decrease in ultraviolet absorption of the effluent was observed. The bound enzyme was then eluted with the equilibration buffer containing 0.5 M KCl. For further purification, the eluted fractions were pooled and applied to a second column. This time elution was performed with a linear gradient of NADPH (0-0.5 mM) in 50 mM phosphate, pH 7.5, resulting in a preparation of at least 98% homogeneity.

When using this resin, in general, any pH lower than 4.0 should be avoided since this resin is made using cyanogen bromide activation chemistry. Also, a pH greater than 9.5 should be avoided for stability of the ADP ligand. One suggestion for cleaning the column is as follows: a low pH wash with 0.1 M sodium acetate, pH 4.5, a water rinse, a high pH wash using 0.05 M Tris, pH 9.0, followed by a water rinse, and then a final wash with 0.5 M sodium chloride. Many times a 5 mM wash of ATP or ADP can also be used to clean the column.

Precautions and Disclaimer

For Laboratory Use Only. Not for drug, household or other uses.

The resin should be swollen at pH 7.0 for at least 15 minutes and washed with buffer before use. The suspension can be stored for 1 year at neutral pH at 4-8 °C in the presence of a bacteriostatic agent (azide or thimerosal). Exposure to pH values greater than 10 may cause hydrolysis of the phosphate groups.

References

1. Schmidt, H.H., et al., Purification of a soluble isoform of guanylyl cyclase-activating-factor synthase. Proc. Natl. Acad. Sci. USA, **88**, 365-369 (1991).
2. Pigiet, V.P., and Conley, R.R., Purification of thioredoxin, thioredoxin reductase, and glutathione reductase by affinity chromatography. J. Biol. Chem., **252**, 6367-6372 (1977).
3. Boggaram, V., et al., Purification of glutathione reductase from porcine erythrocytes by the use of affinity chromatography on 2', 5'-ADP-Sepharose 4B and crystallization of the enzyme. Anal. Biochem., **98**, 335-340 (1979).
4. Carlberg, I., and Mannervik, B., Purification and characterization of glutathione reductase from calf liver. An improved procedure for affinity chromatography on 2',5'-ADP-Sepharose 4B. Anal. Biochem., **116**, 531-536 (1981).

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