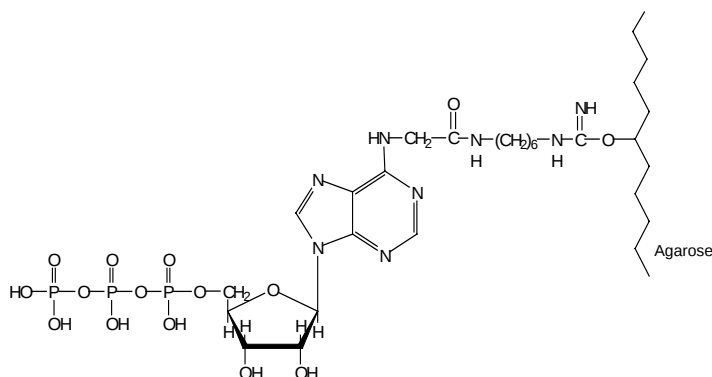


ADENOSINE 5'-TRIPHOSPHATE-AGAROSE

Attachment through N-6

Sigma Prod. No. A-9264

**A9264 ATP Agarose****PRODUCT DESCRIPTION:**

Adenosine 5'-triphosphate (ATP) attached to agarose through the adenine N-6 position has been used to purify β - and γ -glutamate decarboxylase¹ and phosphofructokinase.^{2,3} It also binds citrate kinase,⁴ phosphoglycerate kinase, pyruvate kinase, alcohol dehydrogenase, and lactate dehydrogenase.⁵ This resin does not bind protein kinases,⁶ hexokinase, or albumin.⁵ However, hexokinase, acetate kinase, and pyruvate kinase can use this immobilized ATP as a substrate.^{4,5}

PHYSICAL PROPERTIES:

Appearance: lyophilized powder stabilized with lactose

Matrix: cross-linked 4% beaded agarose

Swelling: 1 g swells to 11-18 ml

Average particle size: 45-165 μ m

Activation: cyanogen bromide

Attachment: N-6 of the adenosine ring

Spacer: 11 atoms

Ligand immobilized: 1-5 μ moles/ml packed resin

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METHOD OF PREPARATION:

The preparation of N⁶-[N-(6-aminohexyl)-carbamyl]-adenosine 5'-triphosphate and its immobilization on cyanogen bromide-activated agarose are described by Lindberg and Mosbach.⁴ The N-(6-aminohexyl)carbamyl group provides an 11-atom spacer arm between ATP and the agarose bead.

STABILITY / STORAGE AS SUPPLIED:

The lyophilized resin should be stored frozen at -20°C. The hydrated resin can be stored refrigerated in water or buffer containing a bacteriostat, such as 0.02% sodium azide or thimerosal. Do not autoclave or freeze the hydrated resin. The resin can be used several times without loss of effectiveness.² However, the ATP will slowly hydrolyze to ADP over time, and under certain conditions this process may be accelerated during usage. Methods for regenerating the resin are given below.

RESIN PREPARATION:

Hydrate the resin by placing it in excess water, approximately 50 ml per g of resin, for at least 30 min. Remove the lactose stabilizer by washing the resin on a Buchner funnel with gentle vacuum using approximately 100 ml of water per g of resin. Do not allow the resin to dry. Resuspend the resin in excess water or starting buffer to pack the column bed.

REGENERATING THE RESIN:

Specifically bound proteins can be eluted with 10-100 mM ATP or ADP. Nonspecifically bound proteins can be eluted with 2 M NaCl or KCl in water or in 7 M urea. Since N⁶-[N-(6-aminohexyl)-carbamyl]-ADP is a substrate for acetate kinase and pyruvate kinase, these enzymes can be used to regenerate ATP from ADP on the resin under conditions favoring the reverse reaction. Wash the resin with 20 volumes of 50 mM tris HCl buffer, pH 8.2, containing 0.2 mM EDTA and 100 mM KCl. Incubate the resin overnight at 2°C in an ATP regenerating mixture containing 0.2 mM phosphoenol pyruvate, pyruvate kinase (10 units per ml of resin), 5 mM MgCl₂, 0.2 mM EDTA and 100 mM KCl in 50 mM tris HCl buffer, pH 8.2. Wash the resin with 25 column volumes of 2 mM ATP in 2 M KCl and reequilibrate the resin with 25 column volumes of sample buffer.² Alternatively, the ATP regenerating mixture can contain 20 mM acetyl phosphate, acetate kinase (6.8 units per ml of resin), and 3 mM MgCl₂ in 100 mM tris HCl, pH 7.6. If acetate kinase is used, prewash the resin with 25 column volumes of 100 mM tris HCl, pH 7.6, but use the same final wash and reequilibration steps as given for pyruvate kinase.⁴

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APPLICATIONS:

Ramadoss, et al.,² purified phosphofructokinase to homogeneity by loading the partially-purified enzyme on this resin in 50 mM tris phosphate buffer, pH 8.0, containing 0.2 mM EDTA, and 5-10 mM DTT. The column was washed with 20-30 volumes of starting buffer, and the enzyme was eluted with starting buffer containing 25-50 μ M fructose 6-phosphate and 25 μ M ADP, which form a dead-end complex with the enzyme.⁷ Alternatively, it could be eluted with 250 μ M ATP. The enzyme did not elute with either 100 μ M ADP or 1 mM fructose 6-phosphate alone, nor did it elute with 1 mM fructose 1,6-diphosphate or 25 μ M ATP. The flow rate for elution was 15 ml/hr. Hand and Somero³ eluted phospho-fructokinase with 140 μ M fructose 6-phosphate and 100 μ M ADP, then incubated the eluate for 5 min at 65°C in 100 mM phosphate buffer, pH 8.0, containing 1 mM EDTA and 5 mM DTT, to obtain a homogeneous enzyme.

Wu and Martin¹ purified glutamate decarboxylase (GAD) by loading the partially-purified enzyme on the resin in 50 mM imidazole-acetate buffer, pH 7.2. The column was washed with several column volumes of the same buffer. α -GAD eluted in the buffer wash. β - and γ -GAD eluted together with either 10 mM ATP, 15 mM ADP, 25 mM AMP, 140 mM inorganic phosphate, or 4 mM pyridoxal phosphate. In the presence of 1 mM inorganic phosphate, GAD eluted with 2.5 mM pyridoxal phosphate. The flow rate was 16 ml/hr. GAD was enzymatically active while bound to ATP agarose and eluted as the holoenzyme.

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