Product Information

Adenosine 5’-triphosphate Agarose

A2767

Product Description

Adenosine 5’-triphosphate-Agarose (5’-ATP-agarose) is a conjugate of 5’-ATP to cross-linked 4% beaded agarose (activated by cyanogen bromide), via the C-8 atom of 5’-ATP. A 9-atom matrix spacer arm connects the ATP and the agarose.

5’-ATP-agarose is useful in affinity chromatography for purification of various proteins and enzymes,[1] such as:

- Cyclin-dependent kinase 2 (CDK2)[2]
- Heat shock proteins[3,4]
- Cryptochromes[5]

Preparation Instructions

- The 5’-ATP-agarose resin may be hydrated with excess water, such as 30-50 mL per gram of resin, with adjusted volumes as needed based on the observed degree of swelling, for at least 30 minutes.
- The lactose stabilizer may be removed by washing the resin on a Buchner funnel with gentle vacuum, using 50-100 mL of water per gram of resin.
- Do not allow the resin to dry.
- The washed resin may then be resuspended in excess water or starting buffer, to pack the column bed.

Storage/Stability

- The lyophilized 5’-ATP-agarose resin should be stored at -20 °C.
- Hydrated 5’-ATP-agarose resin can be stored refrigerated in neutral pH buffer (commonly in the pH range 7.0-8.5) that contains a bacteriostat, such as 0.02% sodium azide or 20% ethanol.
- Do not autoclave or freeze the hydrated resin.

Regeneration Procedure

With time, the ATP will slowly hydrolyze to ADP. This process may be accelerated under certain conditions during usage. To regenerate the resin, either acetate kinase or pyruvate kinase may be used to regenerate ATP from ADP on the resin, by analogy of reactivity of these enzymes with N^6-[N-(6-aminohexyl)-carbamyl]-ADP, but under conditions that favor the reverse reaction.

A protocol for use of pyruvate kinase in the ATP-regeneration protocol is as follows:

1. Wash the resin with 20 volumes of 50 mM Trizma®-HCl (pH 8.2), containing 0.2 mM EDTA and 100 mM KCl.
2. 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 $MgCl_2$, 0.2 mM EDTA and 100 mM KCl in 50 mM Trizma®-HCl (pH 8.2).
3. Wash the resin with 25 column volumes of 2 mM ATP in 2 M KCl.
4. Re-equilibrate the resin with 25 column volumes of sample buffer.[6]

Alternatively:

- The ATP-regenerating mixture can contain 20 mM acetyl phosphate, acetate kinase (6.8 units per mL of resin), and 3 mM $MgCl_2$ in 100 mM Trizma®-buffer (pH 7.6).
- With acetate kinase, prewash the resin with 25 column volumes of 100 mM Trizma®-HCl (pH 7.6).
- The same final wash and re-equilibration steps for pyruvate kinase may be used, as given, for acetate kinase.[7]

Procedure

Equilibration of the resin may be accomplished with various neutral aqueous buffers. Examples include:

- 10 mM HEPES (pH 7.5), 25 mM NaCl, 0.5 mM DTT, 1 mM EDTA, and 10% glycerol[2]
- 25 mM HEPES (pH 7.6), 50 mM KCl, 2.5 mM[3] or 5 mM[4] $MgCl_2$, 10 mM 2-mercaptoethanol, and 1 mM EDTA
- 25 mM HEPES (pH 7.4), 150 mM NaCl; 1 mM DTT, and 60 mM $MgCl_2$[5]

Specifically bound proteins are then eluted with 10-100 mM ATP or ADP included in the previously used equilibration buffer. To aid in elution, increasing amounts of salt (e.g. KCl, NaCl) may also be used, in successive formulations of the elution buffer. The presence of such salts helps to reduce non-specific electrostatic interactions with the agarose.

Nonspecifically bound proteins can be eluted with 2 M NaCl or KCl in water or in 7 M urea.

References

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