PROTOCOL

ATP Agarose

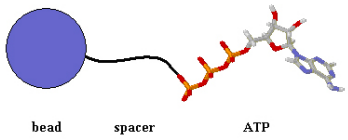
Applicable to: 510-0002 2ml – ATP Agarose high (8-12µmol/ml)
510-0005 5ml – ATP Agarose high (8-12µmol/ml)

INTRODUCTION

Affinity resins have been widely used for the purification of enzymes that bind nucleotides and related molecules. Resins in which ATP is linked via the -phosphate have been valuable in identifying proteins in the purine-binding proteome, which includes kinases, heat shock proteins and other ATP-binding proteins.

ATP Agarose comprises ATP attached to agarose beads. A long hydrophilic spacer (14-atom) is used to minimize unwanted hydrophobic interactions and to facilitate unhindered interactions with biomolecules. The ATP is coupled via its -phosphate group which means that the resin is resistant to phosphatases found in many crude tissue extracts.

Figure 1. Schematic diagram of ATP Agarose



STORAGE AND SHIPPING

The resin is supplied as 50% (v/v) slurry in 10mM Tris/300mM NaCl/1mM EDTA, pH 8.0. It is shipped at ambient temperature but should be stored at 4°C.

MATERIALS REQUIRED (BUT NOT SUPPLIED)

For small sample volumes you may need only a microfuge and 1.5ml tubes. For larger volumes (up to 20ml) the purification of binding proteins is conveniently carried out using disposable polypropylene columns. A simple mixing device (e.g. rotary shaker or end-over-end mixer) may also be useful.

OVERVIEW OF PROCEDURE

The ATP Agarose is added to a crude protein extract and the suspension is gently mixed. After a period of incubation the resin is transferred to a disposable column and washed to remove non-bound or loosely adsorbed material. Finally, the column is eluted with buffer containing a competing ligand.

Since ATP Agarose may be used to capture many ATP-binding proteins the instructions below provide only general guidance on the use of resin. You may need to modify the conditions to facilitate the binding of your particular biomolecule of interest.

BUFFERS

For simplicity we would recommend that you start with the same buffer for the equilibration, binding and wash steps. The elution buffer is prepared by adding a competing ligand.

Types of buffers

The buffer and pH must be compatible with the biomolecule of interest. Tris (pH 7.5-8.5) and Hepes (pH 7.0-8.0) are commonly used but other buffers may also be suitable.

Metal ions

ATP-binding proteins usually recognize ATP-magnesium ion complexes rather than free ATP. It is usual to include $MgCl_2$ (at least 10mM) in all column buffers to facilitate metal-dependent interactions with the resin.

Salts

To prevent non-specific electrostatic interactions with the matrix include 100mM–500mM NaCl, KCl or other salt in the buffer.

Thiols

Thiols are often included in buffers to prevent oxidation of cysteine residues. A final concentration of 1mM DTT is commonly used. DTT is not stable and should be added to the buffer immediately before use.

Protease inhibitors

Protease inhibitors (e.g. PMSF, benzamidine) may or may not be required, depending on the sensitivity of the protein of interest to proteolysis. It is also advisable to carry out the binding and wash steps in a cold room or fridge using ice-cold buffers.

Detergents

Detergents (e.g. Triton X-100) are sometimes used to prevent non-specific hydrophobic interactions. Since the resin and spacer are hydrophilic a detergent may not be necessary. However, if a detergent is required try relatively low concentrations (0.02-0.1%) in the first instance.

CHROMATOGRAPHY STEPS

Make sure the resin has been fully equilibrated with the column equilibration buffer before commencing the purification procedure. Dialyse or desalt the sample into the same buffer before application to the resin.

Binding step

If you do not have access to an automated chromatography system a batch-binding method may be used. Protein samples with volumes of 0.5-1.0ml should be incubated in 1.5ml tubes with 50-100µl of ATP agarose resin. For larger sample volumes the incubation should be carried out in 10ml, 30ml or 50ml tubes (or in a capped disposable column with an integral upper reservoir). Allow at least 1 hour at 4°C for binding to take place, and agitate the sample at regular intervals to prevent settling of the resin.

If you have a pump system the recommended flow rate in the first instance is 0.1-0.25ml/min for columns that are 1-5ml in size, though you may wish to explore higher flow rates especially if the volume of material to be processed is large.

Wash step

If incubations have been carried out in small tubes, the resin should be subjected to five or more cycles of washing and centrifugation (e.g. in a microfuge for 3-4 seconds) using ice-cold buffers. On a larger scale it is easier to transfer the suspension to a disposable polypropylene column and to allow the non-bound material to drip through under the force of gravity. Add the wash buffer carefully down the inner surface of the column and try not to disturb the resin otherwise the wash buffer will mix with the non-bound material, leading to less efficient washing of the resin. It is important to remove all of the non-bound material prior to elution. The absence of protein in the washes is easily verified with a dye-based protein detection reagent (e.g. Bradford reagent) or with a UV monitor.

Elution step

It is important to appreciate in affinity chromatography that the eluting ligand (competing ligand or 'displacer') does not usually drive the bound protein from the resin; rather, it associates with proteins that dissociate from the resin and prevents their rebinding. The concentration of the displacer has to be sufficiently high to compete with any unoccupied ligand sites on the resin and sufficient time has to be allowed for dissociation to take place. The high ligand density of the resin (8-12µmol/ml; 8-12mM) means it would need a high ligand concentration for successful elution. If ATP is used as the competing ligand the concentration in the range 5-10mM is a useful starting point.

For experiments carried out in 1.5ml tubes, the elution buffer (0.25-1.0ml) is added to the resin. After >30 minutes the resin is centrifuged and the supernatant fraction is carefully removed. If a drip column format is used, the displacer is allowed to pass into the column bed and the flow is then halted (e.g. by capping the column outlet). After a period of equilibration (15-30 minutes) the dissociated proteins are flushed out by application of more elution buffer. This step can be repeated until protein is absent from the eluted fractions. If a pump is available the column can be eluted using continuous flow at a rate of 0.05-0.1ml/min, but it may be necessary to reduce the flow rate (or switch off the pump for a period of time) if the protein elutes in a relatively large volume.

COLUMN REGENERATION

After each run, wash the column with a neutral buffer containing 1M NaCl and then re-equilibrate with 10mM Tris/300mM NaCl, 1mM EDTA pH 8.0. Do not wash the column with strong acid or base. For long-term storage add a preservative (e.g. 0.1% sodium azide).

RELATED PRODUCTS

Description	Prod. Code
Control Resin	520-0002

This is agarose with no nucleotide bound. It should be loaded, washed and eluted in exactly the same way as the ATP Agarose.

FAQ

Q1. What is the best buffer to use?

There is no 'right' answer here. In the absence of any information on the binding requirements of the protein(s) of interest a good starting point is a buffer containing 20mM Hepes, 100-500mM NaCl (or KCl), 20mM MgCl2 and 1mM DTT, pH 7.5. Alternatively, try experimenting with several buffer conditions using small amounts of resin in 1.5ml tubes. After the wash step, elute with ATP or other competing ligand and analyse the eluted proteins by SDS-PAGE. The purification method can then be scaled up using the preferred buffer conditions.

Q2. Are there any other ways of eluting binding proteins from the ATP Agarose?

Yes. While ATP is the obvious choice, ligands that are structurally related to ATP may be used (e.g. NADH, AMP, adenosine) to elute a specific subset of the ATP-binding proteins. Drugs that are known to bind to ATP-binding proteins might also be used. If preservation of biological activity is not required, aliquots of resin may be boiled with SDS sample buffer prior to gel electrophoresis.