User Guide

IFTA AG
Certified QMS according to
DIN EN ISO 9001:2000-12
Reg. No. IC 03214 034

Jena Bioscience

ATP AffiPur Kit I

For the purification of ATP binding proteins using AP-ATP-Agarose

Cat. No.	Amount
AK-103S	1 Kit
AK-103L	1 Kit

Kit Contents

Component	S Kit	L Kit
Aminophenyl-ATP-Agarose, C_{10}-spacer (Cat. No. AC-101) pre-swollen in 20% ethanol	1 ml	5 ml
PBS Tablets (Cat. No. AK-102P)	10 tabs	50 tabs
200 mM Sodium Orthovanadate, activated, pH 10.0 (Cat. No. AK-102V)	100 µl	500 µl
100x Protease Inhibitor Mix (Cat. No. AK-102I)	0.5 ml	5x 0.5 ml
5x Binding Buffer (contains Hepes, NaCl, $MgCl_2$, and 0.25% NP-40*) (Cat. No. AK-102B)	15 ml	75 ml
5x Wash Buffer (contains Hepes, NaCl, $MgCl_2$, and 0.25% NP-40*) (Cat. No. AK-102W)	10 ml	50 ml
5x Elution Buffer (contains Hepes, ATP, and 0.25% NP-40*) (Cat. No. AK-102E)	2 ml	10 ml

Additional Reagents and Material required

Microcentrifuge vials, Microcentrifuge,
H_2O, 500 mM EDTA, 100 mM DTT, 1 M DTT

* NP-40 can interfere in some protein quantification assays.

Storage and Stability

Store ATP-Agarose, Binding Buffer and Wash Buffer at 4°C. Store Protease-Inhibitor-Mix, Elution Buffer, and Sodium Orthovanadate at -20°C. PBS tablets can be kept at room temperature. Quality guaranteed for 12 months.

Introduction

A characteristic of many proteins is their ability to bind specific small molecules (ligands) non-covalently with high affinity. This protein-ligand interaction can be used for rapid purification of a protein by affinity chromatography. In this technique, a ligand is immobilized onto the surface of a matrix (e.g. Agarose), which is incubated with a protein mixture to be purified. The protein of interest will bind to its ligand whereas other contaminants will not. These contaminants can be washed off, and the protein of interest can be eluted by an excess of free ligand in the elution buffer.

A ligand commonly used for this technique is ATP (adenosine-5'-triphosphate) since a very large number of proteins such as kinases, motor proteins, and chaperones bind ATP with high affinity. There is however, a fundamental problem with using ATP in affinity chromatography: For attachment to a matrix ATP needs to be chemically modified with a linker (Fig. 1). This linker may interfere with the protein-ATP interaction and thereby reduce the binding.

This problem can usually be circumvented by attaching ATP at a different position at the adenine base, the sugar or the phosphate moiety. Each of these linkage strategies has a characteristic effect upon protein-ATP interactions, and all have been applied to the purification of a variety of distinct proteins (Fig. 1).

User Guide

IFTA AG
Certified QMS according to
DIN EN ISO 9001:2000-12
Reg. No. IC 03214 034

Jena Bioscience

ATP AffiPur Kit I

For the purification of ATP binding proteins using AP-ATP-Agarose

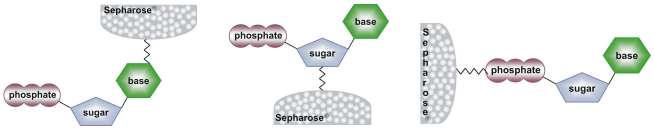

Fig. 1: Possible linkages of ATP to Agarose through modification of base, sugar, or phosphate moiety

To determine the most suitable ATP-Agarose for the purification of a particular protein, the ATP Affinity Test Kit (Cat.# AK-102) can be used. This kit contains four different ATP-Agaroses plus the corresponding buffers. The ATP AffiPur Kits I – IV contain one of these materials each, suitable for the purification of ATP binding proteins in preparative scale.

Kit description

The ATP AffiPur Kit I contains Aminophenyl-ATP-Agarose, C_{10}-spacer (***AP-ATP-Agarose***) and the main components required for the purification of ATP binding proteins.

In ***AP-ATP-Agarose***, the ATP molecule is linked to the matrix via its phosphate moiety (Fig. 2). This material can be used to purify ATP binding proteins from any protein solution.

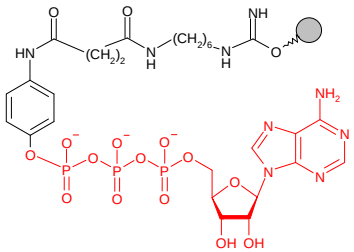

Fig. 2: Structure of the ATP-Agarose material

All ATP-Agaroses (also available in bulk amounts) and the other kit components can be ordered separately (see www.jenabioscience.com for details).

Properties of the ATP-Agarose material

Bead size	45 - 165 µm
Degree of substitution	20 µmol AP-ATP/ml Agarose
pH stability (short term)	4 - 9
pH stability (long term)	7.5
Chemical stability	Stable to all solutions commonly used in gel filtration including 8 M urea and 6 M guanidine hydrochloride. Not stable in organic solvents!

Table 1: Properties of ***AP-ATP-Agarose***

Experimental Protocol

Sample preparation

Prepare crude protein solution using appropriate buffer conditions to maintain native protein structures and functionally active proteins. All ATP-Agaroses are compatible with detergents. Take an aliquot of the protein solution for later analysis.

ATP depletion

For a successful purification it is necessary to remove free endogenous ATP from the protein solution by dialysis or gel filtration before loading onto ATP-Agarose. For dialysis 3 buffer changes are recommended with a sample-to-volume ratio of 1:100.

Dialysis

1. Add 1 PBS Tablet to 500 ml of de-ionized water and stir until dissolved.

ATP AffiPur Kit I

For the purification of ATP binding proteins using AP-ATP-Agarose

2. Add 1 ml of 500 mM EDTA and 0.5 ml of 1 M DTT (final concentration of 1 mM each).
3. Dialyse the protein solution at 4°C for 4-6 h.
4. Exchange buffer two times.

Sample preparation

Before incubation of the ATP-depleted solution with ATP-Agarose add

1. 1 volume of 5x Binding Buffer per 4 volumes of the dialysed solution.
2. 10 µl of 100 mM DTT per ml of the protein solution from step 1.
3. 10 µl of 100x Protease-Inhibitor-Mix per ml of the protein solution from step 2.

Protein Purification

The following protocols are optimized for the use of 50 µl ATP-Agarose. For larger or smaller amounts, please increase or decrease the amount of buffers accordingly.

Buffer preparation

For 5 ml 1x Wash Buffer please add in the following order:

1 ml	5x Wash Buffer
3.9 ml	deionized water
5 µl	200 mM Sodium Orthovanadate
50 µl	100 mM DTT
50 µl	100x Protease Inhibitor Mix

For 500 µl 1x Elution Buffer please add in the following order:

100 µl	5x Elution Buffer
400 µl	deionized water
0.5 µl	200 mM Sodium orthovanadate
5 µl	100 mM DTT
5 µl	100x Protease Inhibitor Mix

Mix and keep on ice!

Equilibration of the ATP-Agarose

1. Add 500 µl of 1x Wash Buffer to 50 µl of ATP-Agarose.
2. Mix by vortexing and spin at 1000 x g for 1 min.
3. Remove Buffer and discard.
4. Repeat step 1 - 3 two more times.

Binding, Washing, and Elution

1. Add dialyzed protein solution to the equilibrated ATP-Agarose.
2. Incubate at 4°C for 2 - 3 h by slight agitation.
3. Spin at 1000 x g for 1 min. Transfer the supernatant to a fresh tube for later analysis.
4. Resuspend pellet in 1 ml ice-cold 1x Wash Buffer and spin at 1000 x g for 1 min. Transfer the supernatant to a fresh tube for later analysis. Wash two more times.
5. Add 150 µl ice-cold 1x Elution Buffer (2-3 bead volumes) to the ATP-Agarose and incubate at 4°C for 20 min while slightly agitating.
6. Spin at 1000 x g for 1 min. Transfer the elution fraction to a fresh tube for later analysis.
7. Repeat steps 5 - 6 two more times.

Analyze all fractions by SDS-PAGE in order to identify the protein of interest. Larger volumes of chromatography material are available from www.jenabioscience.com.

User Guide

IFTA AG
Certified QMS according to
DIN EN ISO 9001:2000-12
Reg. No. IC 03214 034

Jena Bioscience

ATP AffiPur Kit I

For the purification of ATP binding proteins using AP-ATP-Agarose

Selected references

Rachidi *et al.* (2014) Pharmacological assessment defines *Leishmania donovani* casein kinase 1 as a drug target and reveals important functions in parasite viability and intracellular infection. *Antimicrobial Agents and Chemotherapy* **58**:1501.

Haystead *et al.* (1993) Gamma-phosphate-linked ATP-Sepharose for the affinity purification of protein-kinases - rapid purification to homogeneity of sceletal-muscle mitogen-activated protein-kinase. *Eur. J. Biochem.* **214 (2)**:459.

Jenö *et al.* (1989) Purification and Charaterization of a 40 S Ribosomal Protein S6 Kinase from Vanadate-stimulated Swiss 3T3 Cells. *J. Biol. Chem.* **264**:1293.

McNutt *et al.* (1981) Comparison of cell peripheries in the human colonic adenocarcinoma cell lines SW480 and SW620 grown in floating chamber culture, cover slip culture, athymic (nude) mice, and BALB/c mice. *Lab. Invest.* **44**:309.

Trayer *et. al.* (1974) Affinity Chromatography of Nicotinamide Nucleotide-Dependent Dehydrogenases on Immobilized Nucleotide Derivates. *Biochem. J.* **139**:609.

Scherer *et al.* (1954) Studies on the propagation in vitro of poliomyelitis viruses. IV. Viral multiplication in a stable strain of human malignant epithelial cells (strain HeLa) derived from an epidermoid carcinoma of the cervix. *J. Exp. Med.* **97**:695.

Associated products available from Jena Bioscience

For detailed information please view the sections on

www.jenabioscience.com

Aminophenyl-ATP-Agarose, C_{10}-spacer pre-swollen in 20% ethanol	Cat.# AC-101
8-[(6-Amino)hexyl]-amino-ATP-Agarose pre-swollen in 20% ethanol	Cat.# AC-127
N^6-(6-Amino)hexyl-ATP-Agarose pre-swollen in 20% ethanol	Cat.# AC-129
2'/3'-EDA-ATP-Agarose pre-swollen in 20% ethanol	Cat.# AC-131
PBS Tablets	Cat.# AK-102P
200 mM Sodium Orthovanadate, activated, pH 10.0	Cat.# AK-102V
100x Protease Inhibitor Mix	Cat.# AK-102I
5x Binding Buffer	Cat.# AK-102B
5x Wash Buffer	Cat.# AK-102W
5x Elution Buffer	Cat.# AK-102E
ATP AffiPur Kit II (For the purification of ATP binding proteins using 8AH-ATP-Agarose)	Cat.# AK-104
ATP AffiPur Kit III (For the purification of ATP binding proteins using 6AH-ATP-Agarose)	Cat.# AK-105
ATP AffiPur Kit IV (For the purification of ATP binding proteins using EDA-ATP-Agarose)	Cat.# AK-106