AK1

PDB:1Z83

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:NP_000467
Entry Clone Source:TCK
SGC Clone Accession:
Tag: Tag sequence: MHHHHHHSSGVDLGTENLYFQ*S(M) TEV-cleavable (*) N-terminal his6 tag.
Host:BL21 (DE3)

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfqSMEEKLKKTNIIFVVGGPGSGKGTQCEKIVQKYGYTHLSTGDLLRSEVSSGSARGKKLSEIMEKGQ
LVPLETVLDMLRDAMVAKVNTSKGFLIDGYPREVQQGEEFERRIGQPTLLLYVDAGPETMTQRLLKRGETSGRVDDNEETIKKRLET
YYKATEPVIAFYEKRGIVRKVNAEGSVDSVFSQVCTHLDALLN

Vector:pLIC-SGC

Growth

Medium:
Antibiotics:
Procedure: Grow starter cultures from freshly transformed colonies in 10 mL LB, 0.1 mg/mL amp. This started culture was diluted 1:1000 in fresh media and was grown at 37 ºC to a OD_{600} of 0.3 and than transferred to 18 ºC. Expression was induced at an OD_{600} of 0.6 using 1 mM IPTG. Cells were harvested after 4 hrs by centrifugation, transferred to 50 mL tubes, and frozen in -20 ºC.

Purification

Procedure
Column 1 : DE52/Ni-NTA

Loading buffer: 50 mM Hepes, pH 7.5, 500 mM NaCl, 10 mM imidazole, 0.5 mM TCEP, 5% glycerol. Wash buffer: 50 mM Hepes, pH 7.5, 500 mM NaCl, 50 mM imidazole, 5% glycerol. Elution buffer: 50 mM Hepesl, pH 7.5, 500 mM NaCl, 250 mM imidazole, 0.5 mM TCEP, 5% glycerol.

Procedure: Gravity feed chromatography. Sample applied to a 10 mL DE-52 column and washed through with 50 ml loading buffer. The flow through was applied to a 1 ml Ni-NTA column, the Ni-NTA column was washed with 2x10mL of wash buffer and eluted with elution buffer in 5 mL aliquots (Step elution using 50, 100, 150, 200 and 250 mM imidazole in the Elution Buffer).

Enzymatic treatment: Treated the IMAC elution(s) with TEV protease overnight.

SEC: Procedure : AKTA-prime. Fractions containing AK1A collected from IMAC and treated with TEV protease overnight were concentrated to 1.5ml and directly applied to a S75 16/60 column equilibrated in 10 mM Hepes pH 7.5, 100 m NaCl. Flow rate 1mL/min.

Concentration: Centricons 10 kDa cut off.

Extraction

Procedure
Extraction buffer: 50 mM Tris-HCl, pH 7.5, 500 mM NaCl. The cell pellets (4 gm wet wt) were re-suspended in 50 mL extraction buffer containing a Protease Inhibitor Coctail tablet (Roche), and lysed by a high pressure cell disrupter. Supernatant was centrifuged for 30 minutes at 17 rpm in a JA 25.5 rotor.
Concentration:
Ligand
MassSpec:
Crystallization: Crystals were grown at 4 ºC in 500 nL sitting drops mixing 250 nl of AK1 (14 mg/mL in 10mM Hepes pH 7.5, 100mM NaCl ,10mM DTT) with 250 nL of a solution containing 20% PEG 550, 0.005 M ZnSO 4 , MES pH 6.5 and 1mM AP 5 A (Diadenosine pentaphosphate pentalithium salt - CAS # 94108-02-8)
NMR Spectroscopy:
Data Collection:
Data Processing: