Entry Clone Source: TKC

Entry Clone Accession: n/a

SGC Construct ID: AK1A-c001

GenBank GI number: gi|4502011

Final protein sequence:

mhhhhhhssgvdlgtenlyfqSMEEKLKKTNIIFV
VGGPGSGKGTQCEKIVQKYGYTHLSTGDLLRSEVS
SGSARGKKLSEIMEKGQLVPLETVLDMLRDAMVAK
VNTSKGFLIDGYPREVQQGEEFERRIGQPTLLLYV
DAGPETMTQRLLKRGETSGRVDDNEETIKKRLETY
YKATEPVIAFYEKRGIVRKVNAEGSVDSVFSQVCT
HLDALLN

Vector: pLIC- SGC1. Details [PDF]; Sequence [FASTA] or [GenBank]

Tags and additions: mhhhhhhssgvdlgtenlyfq*s(m) TEV-cleavable (*) N-terminal his6 tag.

Host: BL21 (DE3)

Growth medium, induction protocol: 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°C to a OD_{600} of 0.3 and than transferred to 18°C. Expression was induced at an OD_{600} of 0.6 using 1 mM IPTG. Cells were harvested after 4h by centrifugation, transferred to 50-ml tubes, and frozen in -20oC.

Extraction buffer, extraction method: Extraction buffer: 50 mM Tris-HCl, pH 7.5, 500 mM NaCl. The cell pellets (4 gr 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

Column 1 : DE52/Ni-NTA

Buffers: Loading buffer: 50 mM Hepes, pH 7.5, 500 mM NaCl, 10 mM imidazole, 5% glycerol. **Wash buffer:** 50 mM Hepes, pH 7.5, 500 mM NaCl, 20 mM imidazole, 5% glycerol. **Elution buffer:** 50 mM Hepesl, pH 7.5, 500 mM NaCl, 50-250 mM imidazole, 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.

Column 2: 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.

Mass spec characterization: LC- ESI -MStof confirmed the correct mass expected for this construct.

Protein concentration: Centricons 10 kDa cut off

Crystallization: 1Z83: Crystals were grown at 4°C in 500nl 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). **2C95:** Crystal were obtained at 4 °C in sitting drops by mixing 100 nl AK1 [20

mg/ml, in 10mM HEPES pH=7.5, 100mM NaCl, 10mM DTT, 1 mM $ZnSO_4$, 1 mM AP4A (diadenosine tetraphosphate)] with 50 nl of the reservoir solution 2.4 M Na-malonate pH=7.0.
Data Collection: 1Z83: Resolution: 1.9Å, **X-ray source:** SLS X06SA, single wavelength. **2C95: Resolution:** 1.7Å, **X-ray source:** rotating anode (Rigaku FR-E SuperBright), single wavelength.