AK4

PDB:2AR7

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:NM_013410.2
Entry Clone Source: Toronto sick kid hospital
SGC Clone Accession:
Tag: Tag sequence: mhhhhhhssgvdlgtenlyfq*s(m). TEV-cleavable (*) N-terminal his6 tag.
Host:BL21 (DE3)

Construct

Prelude:
Sequence:

```
mhhhhhhssgvdlgtenlyfqsMASKLLRAVILGPPGSGKGTVCQRIAQNFGLQHLSSGHFLRENIKASTEVGEMAKQYIEKSLLVP
DHVITRLMMSELENRRGQHWLLDGFPRTLGQAEALDKICEVDLVISLNIPFETLKDRLSRRWIHPPSGRVYNLDFNPPHVHGIDDVT
GEPLVQQEDDKPEAVAARLRQYKDVAKPVIELYKSRGVLHQFSGTETNKIWPYVYTLFSNKITPIQSKEAYL
```

Vector:pLIC- SGC1

Growth

Medium:
Antibiotics:
Procedure: Grow starter cultures from freshly transformed colonies in 10 ml LB, 0.1 mg/ml ampiciline. This started culture was diluted 1:1000 in fresh media and was grown at 37°C to an OD600 of 0.4 and than transferred to 18°C. Expression was induced at an OD600 of 0.6 - 0.7 using 1 mM IPTG (final concentration). Cells were harvested after 4h by centrifugation (15min, 6000rpm on a JLA 8.100 rotor), transferred to 50-ml tubes, and frozen at -20°C.

Purification

Procedure
Column 1: A DE52 column (10gr in 100ml of 2.5M NaCl) was equilibrated with 100ml of Loading Buffer. A 5ml NiNTA column was equilibrated with 20ml of Loading Buffer.

Buffers: Loading buffer: 50 mM Hepes, pH 7.5, 500 mM NaCl, 5% glycerol. Wash buffer: 50 mM Hepes, pH 7.5, 500 mM NaCl, 20 mM imidazole, 5% glycerol. Elution buffer: 50 mM Hepes, pH 7.5, 500 mM NaCl, 50-250 mM imidazole (step elution), 5% glycerol.

Procedure: A DE52 column (10gr suspended in 100ml of 2.5M NaCl) was equilibrated with 100ml of Loading Buffer. A 5ml NiNTA column was equilibrated with 20ml of loading buffer. The lysed sample was applied to the DE52 column and washed through with 50 ml loading buffer. The flow through was applied to the 5 ml Ni-NTA column which 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).

Column 2: SEC

Buffers : Gel Filtration Buffer: 10mM HEPES, pH 7.5, 100mM NaCl

Procedure : AKTA-prime. Fractions containing AK3A collected from IMAC and treated with TEV protease overnight (identified by SDS PAGE) were concentrated to about 1.5ml and directly applied to a S75 16/60 column equilibrated in 10 mM Hepes pH 7.5, 100 m NaCl. The flow rate was 1ml/min and the pure protein eluted at 60-70min.

Enzymatic treatment: Treated the IMAC elution(s) with TEV protease overnight at 4°C.

Extraction

Procedure
50 mM HEPES, pH 7.5, 500 mM NaCl, 5% Glycerol. The cell pellets (5 gr wet wt) were re-suspended in 50 ml extraction buffer containing a Protease Inhibitor cocktail tablet (Roche), and lysed in a high pressure cell disrupter. The supernatant was centrifuged for 30 minutes at 35k x g in a JA 25.5 rotor at 4°C.
Concentration:
Ligand
MassSpec:
Crystallization: Crystals were grown at 4°C in 200nl sitting drops mixing 50nl of AK3A (15 mg/ml in 10mM Hepes pH 7.5, 100mM NaCl ,10mM DTT) with 150nl of a solution containing 0.7M sodium succinate.
NMR Spectroscopy:
Data Collection:
Data Processing: