AK3A

PDB:2BBW

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:mhhhhhhssgvdlgtenlyfq*s(m). TEV-cleavable (*) N-terminal his6 tag.
Host:BL21 (DE3)

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfqsmASKLLRAVILGPPGSGKGTVCQRIAQNFGLQHLSSGHFLRENIKASTEVGEMAKQYIEKSLLVP
DHVITRLMMSELENRRGQHWLLDGFPRTLGQAEALDKICEVDLVISLNIPFETLKDRLSRRWIHPPSGRVYNLDFNPPHVHGIDDVT
GEPLVQQEDDKPEAVAARLRQYKDVAKPVIELYKSRGVLHQFSGTETNKIWPYVYTLFSNKITPIQSKEAYL

Vector:pLIC-SGC

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 DE-52 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 AK4 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.

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 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 AK4 (15 mg/ml in 10mM Hepes pH 7.5, 100mM NaCl ,10mM DTT containing 1 mM Gp5G) with 150nl of a solution containing 0.7M sodium succinate. Cryo protection was achieved by adding to the crystallization mix sucrose 1.2 M sodium succinate solution.
NMR Spectroscopy:
Data Collection: Resoultion: 2.05 Å; X-ray source: rotating anode (Rigaku FR-E SuperBright), single wavelength.
Data Processing: