ASUCLA

PDB:2J91

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:BC000253
Entry Clone Source:
SGC Clone Accession:
Tag:N-terminal hexahistidine tag with integrated TEV protease cleavage site: mhhhhhhssgvdlgtenlyfq*sm.
Host:

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfqsmAAGGDHGSPDSYRSPLASRYASPEMCFVFSDRYKFRTWRQLWLWLAEAEQTLGLPITDEQIREM
KSNLENIDFKMAAEEEKRLRHDVMAHVHTFGHCCPKAAGIIHLGATSCYVGDNTDLIILRNALDLLLPKLARVISRLADFAKERASL
PTLGFTHFQPAQLTTVGKRCCLWIQDLCMDLQNLKRVRDDLRFRGVKGTTGTQASFLQLFEGDDHKVEQLDKMVTEKAGFKRAFIIT
GQTYTRKVDIEVLSVLASLGASVHKICTDIRLLANLKEMEEPFEKQQIGSSAMPYKRNPMRSERCCSLARHLMTLVMDPLQTASVQW
FERTLDDSANRRICLAEAFLTADTILNTLQNISEGLVVYPKVIERRIRQELPFMATENIIMAMVKAGGSRQDCHEKIRVLSQQAASV
VKQEGGDNDLIERIQVDAYFSPIHSQLDHLLDPSSFTGRASQQVQRFLEEEVYPLLKPYESVMKVKAE

Vector:pNIC-Bsa4

Growth

Medium:
Antibiotics:
Procedure: Cells (BL21(DE3)) from glycerol stocks were grown in 20 mL of Terrific Broth (TB), supplemented with 8 g/L (87 % glycerol), 100 µg/mL Kanamycin, at 30°C over night. The following morning, 20 ml of the over night cultures inoculated 1500 ml TB with 8 g/L (87 % glycerol) , 50 µg/mL kanamycin, and 100 µL BREOX. Cultivation was performed in glass flasks in the Large Scale Expression System (LEX). Cells were grown at 37°C until an OD600 nm of 2 was reached. The cultivations were down-tempered to 18 °C for 1h in a water bath. Expression of target protein was induced by addition of 0.5 mM IPTG and was allowed to continue over night at 18 °C.

Purification

Procedure

Columns:HiTrap Chelating HP 1 ml (IMAC); HiLoadÂ 16/60 Superdex 200 Prep Grade (Gel filtration)

Purification was conducted automatically on an ÄKTA Xpress system operated by UNICORN software at a flow of 0.8 ml/min. Prior to purification columns were equilibrated with IMAC Bind/Wash1 Buffer (HiTrap Chelating HP) and Gel filtration buffer (Superdex 200). The protein sample was loaded on the HiTrap Chelating column and was washed with IMAC Bind/Wash1 Buffer followed by IMAC Wash2 Buffer. Bound protein was eluted from the IMAC columns with 7.5 ml of IMAC Elution Buffer and loaded onto the Gel filtration column. The chromatogram from gel filtration showed one major protein peak that mainly consisted of ASUCLA-h001 as shown by SDS-PAGE analysis. TCEP was added to the pooled protein peak to a final concentration of 2 mM. The protein started to precipitate after pooling and was centrifuged for 1h at 49 000 x g, 4°C, then concentrated and centrifuged again for 15 min at 24 100 x g, 4 degC to 11.98 mg/ml and stored at -80 degC.

Extraction

Procedure

Concentration:
Ligand
MassSpec:
Crystallization: Crystallization was performed using the vapour diffusion method with hanging drops containing 0.8 microL of protein solution (5.5 mg/mL) + 10mM AMP and 0.8 microL well solution (20% PEG 3350, 200 mM Mg-formate), incubated at 20 degC.
NMR Spectroscopy:
Data Collection:
Data Processing: