CBS

PDB:4UUU

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:IMAGE:3028099
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal, TEV protease cleavable hexahistidine tag
Host:

Construct

Prelude:
Sequence:

```
"MHHHHHHSSGVDLGTENLYFQSMKPWWWHLRVQELGLSAPLTVLPTITCGHTIEILREKGFDQAPVVDEAGVILGMVTLGNMLSSL
LAGKVQPSDQVGKVIYKQFKQIRLTDTLGRLSHILEMDHFALVVHEQQRQMVFGVVTAIDLLNFVAAQE"MHHHHHHSSGVDLGTEN
LYFQ*SM is the purification tag plus TEV protease recognition site *.
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Vector:pNIC28-Bsa4

Growth

Medium:
Antibiotics:
Procedure:

Purification

Procedure
Cell LysisCell pellets were dissolved in approximately 60 ml lysis buffer containg EDTA-free protease inhibitors (diluted 1:1000). The resuspended cell pellets were then treated for 1 hour with 2 mg/ml lysozyme at 4 °C and then broken by homogenization. The insoluble cell debris was separated from the soluble fraction by centrifugation. Talon IMACThe clarified soluble fraction was incubated with 3.0 ml pre-equilibriated Talon resin slurry in 50% ratio (v/v) in binding buffer. for 1 hour at 4°C with rotation. After it was centrigued with a low brake speed to pellet the resin. The resulting supernatant was removed and the resin pellet was resuspeneded in the remaining supernatant and was applied to a gravity column. Next the resin was washed with 1 x 10 ml of binding buffer, 5 x 5 ml wash buffer and the protein was eluted with 6 x 3 ml and 2 x 5 ml elution buffer. Size Exclusion Gel FiltrationSuperdex s75 16/60 Gel Filtration. Pooled elution fractions were then applied to the GF column (pre-equlibriated in GF buffer) at 1.0 ml/min. 1.0

ml fractions were collected. Anionic Exchange Chromatography Combined fractions were concentrated to 5.0 ml and then diluted in low salt buffer. This low salt solution was then applied to a pre-equilibrated Resource Q column and then the protein was eluted with a linear gradient of higher salt concentrations using the high salt buffer. Clean fractions were isolated and combined.

Extraction

Procedure
BL21(DE3)-R3-pRARE2A single colony each was used to inoculate 2 x 50 ml TB media with 50 ug/ml kanamycin and 50 ug/ml chloroamphenicol. These were placed in an incubator at 37 °C, 180 rpm and left to grow overnight. The next day these starter cultures were mixed and used to inoculate 3 L of TB media (10 ml starter culture used per 1 L) grown at 37 °C, 180 rpm and supplemented with 50 ug/ml kanamycin and 50 ug/ml chloroamphenicol.. When the OD600 reached 1.8 the cells were induced with 0.1 mM IPTG AT 18 °C and left overnight. Cell harvestCells were harvested by centrifugation at 4,500 g for 15 minutes after which the supernatant was discarded and the cell pellets were stored at -80 °C.
Concentration: To set up plates the sample was concentrated to approximatley 5 mg/ml using a 10 kDa MWCO concentrator.
Ligand
MassSpec: Expected mass: 17425.2 Da Measured mass: 17426.17 Da
Crystallization: Crystals were grown by vapour diffusion in sitting drop at 20°C. A sitting drop consisting of 100 nl protein with 1 mM S-adenosyl-L-methionine and 50 nl well solution was equilibrated against well solution containing 36% PEG550MME -- 0.1M tris pH 7.5 -- 0.2M calcium chloride.
NMR Spectroscopy:
Data Collection: Resolution: 1.9 Å X-ray source: Diamond Light Source beamline IO2
Data Processing: