CBS

PDB:4COO

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:

"MHHHHHHSSGVDLGTENLYFQSMPSETPQAEVGPTGCPHRSGPHSAKGSLEKGSPEDKEAKEPLWIRPDAPSRCTWQLGRPASESP
HHHTAPAKSPKILPDILKKIGDTPMVRINKIGKKFGLKCELLAKCEFFNAGGSVKDRISLRMIEDAERDGTLKPGDTIIEPTSGNTG
IGLALAAAVRGYRCIIVMPEKMSSEKVDVLRALGAEIVRTPTNARFDSPESHVGVAWRLKNEIPNSHILDQYRNASNPLAHYDTTAD
EILQQCDGKLDMLVASVGTGGTITGIARKLKEKCPGCRIIGVDPEGSILAEPEELNQTEQTTYEVEGIGYDFIPTVLDRTVVDKWFK
SNDEEAFTFARMLIAQEGLLCGGSAGSTVAVAVKAAQELQEGQRCVVILPDSVRNYMTKFLSDRWMLQKGFLKEEDLTEKKPWWWHL
RVQELGLSAPLTVLPTITCGHTIEILREKGFDQAPVVDEAGVILGMVTLGNMLSSLLAGKVQPSDQVGKVIYKQFKQIRLTDTLGRL
SHILEMDHFALVVHEQQRQMVFGVVTAIDLLNFVAAQERDQK"MHHHHHHSSGVDLGTENLYFQ*SM is the purification
tag plus TEV protease recognition site *.

Vector:pNIC28-Bsa4

Growth

Medium:
Antibiotics:
Procedure:

Purification

Buffers
Procedure
Buffers Used:Binding/Lysis Buffer: 50 mM Hepes (pH 7.5), 500 mM NaCl, 5% Glycerol, 20 mM Imidazole pH 7.5, 0.01mM TCEP Wash Buffer: 50 mM Hepes (pH 7.5), 500 mM NaCl, 5% Glycerol, 40 mM Imidazole pH 7.5, 0.01mM TCEP Elution Buffer: 50 mM Hepes (pH 7.5), 500 mM NaCl, 5% Glycerol, 250 mM Imidazole pH 7.5, 0.01mM TCEP Gel Filtration Buffer: 10 mM Hepes (pH 7.5), 500 mM NaCl, 5% Glycerol, 0.01mM TCEPNi-NTA 80 mM Buffer: 50 mM Hepes (pH 7.5), 500 mM NaCl, 5% Glycerol, 80 mM Imidazole pH 7.5, 0.01mM TCEP

Low Salt Buffer: 50 mM Hepes (pH 7.5), 50 mM NaCl, 5% Glycerol, 0.01mM TCEP High Salt Buffer: 50 mM Hepes (pH 7.5), 1 M NaCl, 5% Glycerol, 0.01mM TCEP Cell LysisCell pellets were dissolved in approximately 250 ml lysis buffer containg EDTA-free protease inhibitors (1 l per ml of buffer) and 0.1 mM pyridoxal 5'-phosphate. Cell pellets were thoroughly mixed using a homogenizer and then treated for 1 hour with 2 mg/ml lysozyme at 4 °C. Next the resuspended cells were broken by sonication for 10 minutes, 5 seconds on, 5 seconds of at 35 % amplitude. The insoluble cell debris was separated from the soluble fraction by centrifugation at g for 1 hour at 4 CNi-NTA IMACNi-NTA resin (1.25 ml bed volume in a gravity-flow column).The clarified soluble fraction was incubated with 3.0 ml pre-equilibriated resin slurry in 50% ratio (v/v) in binding buffer. for 1 hour at 4°C with rotation after which it spun down at g with a low brake speed. 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 2 x 20 ml of binding buffer, 2 x 20 ml wash buffer and the protein was eluted with 5 x 5 ml elution buffer. Size Exclusion Gel FiltrationSuperdex s200 16/60 Gel Filtration. Elution fractions 1 was then applied to the GF column (pre-equlibriated in GF buffer) at 1.0 ml/min. 1.0 ml fractions were collected. Hexahistidine Tag Removal and Nickel Rebind The N-terminal hexahistidine tag was cleaved by incubating overnight with TEV (20°C) at a 1:20 ratio (w/w). Cleaved protein was purified by applying the treated protein solution onto 0.5 ml of pre-equilibrated Ni-NTA. The column was then washed with 2 x 0.5 ml gel filtration buffer, 2 x 0.5 ml Ni-NTA binding buffer, 2 x 0.5 ml Ni-NTA wash buffer, 2 x 0.5 ml Ni-NTA 80 mM buffer and 2 x 0.5 ml Ni-NTA elution buffer. Cleaved fractions were isolated and combined.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

Buffers
Procedure
Expression strainBL21(DE3)-R3-pRARE2A single colony each was used to inoculate 2 x 50 ml TB media supplemented with 0.002% thiamine-HCl, 0.005% pyridoxine-HCl, 0.2 mM ferric chloride, 0.6 mM ð-aminolevulinic acid, 50 ug/ml kanamycin and 50 ug/ml chloroamphenicol. These were placed in an incubator at 30 °C, 275 rpm and left to grow overnight. The next day these starter cultures were mixed and used to inoculate 6 L of TB media (10 ml starter culture used per 1 L) grown at 30 °C, 275 rpm and supplemented with 0.002% thiamine-HCl, 0.005% pyridoxine-HCl, 0.2 mM ferric chloride, 0.6 mM ð-aminolevulinic acid and containing 50 ug/ml kanamycin. When the OD600 reached 1.8 the cells were induced with 1.0 mM IPTG 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 10.80 mg/ml using a 30 kDa mwco concentrator.
Ligand
MassSpec: Expected mass: 59584.5 Da Measured mass: 59602.6 DaDiscrepency due to water (18 Da).
Crystallization: Crystals were grown by vapour diffusion in sitting drop at 20°C. A sitting drop consisting of 50 nl protein and 100 nl well solution was equilibrated against well solution containing 18% PEG8000 -- 0.1M cacodylate pH 6.8 -- 0.2M calcium acetate
NMR Spectroscopy:
Data Collection: Resolution: 2.20 Å X-ray source: Diamond Light Source beamline IO4
Data Processing: