YMHAH

PDB:2C63

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:YWHAHA-s001
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal hexahistidine tag that was TEV cleaved before crystallisation
Host:BL-21(DE3)R3 - phage resistant

Construct

Prelude:
Sequence:

SMGDREQLLQRARLAEQAERYDDMASAMKAVTELNEPLSNEDRNLLSVAYKNVVGARRSSWRVISSIEQKTMADGNEKKLEKVKAYR
EKIEKELETVCNDVLSLLDKFLIKNCNDFQYESKVFYLKMKGDYYRYLAEVASGEKKNSVVEASEAAYKEAFEISKEQMQPTHPIRL
GLALNFSVFYYEIQNAPEQACLLAKQAFDDAIAELDTLNEDSYKDSTLIMQLLRDNLTLWTSDQQDEEAGEGN

Vector:pNIC28-Bsa4

Growth

Medium:
Antibiotics:
Procedure: Freshly transformed E. coli cells was used to inoculate 2*1 litre of TB plus 50 µg/ml kanamycin. When OD600 reached ~1.0 the temperature was shifted down from 37°C to 25°C for 1 hour before induction with the addition of 1 mM IPTG. Protein expression was allowed to carry on for a futher 4 hours before harvest. The cells were harvested by centrifugation at 4000 rpm for 10 mins and 4°C. The pellets were resuspended in 25 ml of Resuspension Buffer before freezing at -80°C.

Resuspension Buffer (RS): 50 mM Tris pH 8.0, 500 mM NaCl, 5 % Glycerol, 5 mM Imidazole pH 8.0, 0.5 mM TCEP.

Purification

Procedure
Column 1 : Ni-affinity, HisTrap, 1 ml (GE/Amersham)

Buffers: Lysis buffer: 50 mM potassium phosphate buffer pH 7.5, 500 mM NaCl, 10 mM imidazole, 0.5 mM TCEP. Wash buffer: 50 mM potassium phosphate buffer pH 7.5, 500 mM

NaCl, 50 mM imidazole, 0.5 mM TCEP. Elution buffer: 50 mM potassium phosphate buffer pH 7.5, 500 mM NaCl, 250 mM imidazole, 0.5 mM TCEP.

Procedure: The cell extract was loaded on the column at 0.8 ml/min on an AKTA-express system (GE/Amersham). The column was then washed with 10 column volumes of Lysis buffer, 10 column volumes of wash buffer, and then eluted with elution buffer at 0.8 ml/min. The eluted peak of A280 was automatically collected.

Column 2 : Gel filtration, Hiload 16/60 Superdex 200 prep grade 120 ml, Code no. 17-1069-01 Amersham Biosciences

Buffers: GF buffer: 10 mM HEPES pH 7.5, 500 mM NaCl, 0.5 mM TCEP.

Procedure: The eluted fractions from the Ni-affinity Histrap columns were loaded on the gel filtration column in GF buffer at 1.0 ml/min. Eluted proteins were collected in 1 ml fractions. At this stage the purity of the protein was greater than 95 % based on SDS - PAGE analysis. The C-terminal hexahistidine tag was removed by TEV protease treatment. The TEV protease, a hexahistidine-tagged construct, was over-expressed and purified in-house to a final concentration of 2.5 mg/ml.

Extraction

Procedure
1 tablet protein inhibitor in 10ml Resuspension Buffer was added to homogenise for each pellet of 1L growth. Total vol: 45 mls (estimate), Cell breakage: 5 passes through the Emulsiflex C5, Total vol: 50 mls (estimate). Centrifuge for 40 mins at 16000rpm and 4°C. Discard pellet.
Concentration:
Ligand
MassSpec:
Crystallization: Column 1 : Ni-affinity, HisTrap, 1 ml (GE/Amersham)

Buffers: Lysis buffer: 50 mM potassium phosphate buffer pH 7.5, 500 mM NaCl, 10 mM imidazole, 0.5 mM TCEP. Wash buffer: 50 mM potassium phosphate buffer pH 7.5, 500 mM NaCl, 50 mM imidazole, 0.5 mM TCEP. Elution buffer: 50 mM potassium phosphate buffer pH 7.5, 500 mM NaCl, 250 mM imidazole, 0.5 mM TCEP.

Procedure: The cell extract was loaded on the column at 0.8 ml/min on an AKTA-express system (GE/Amersham). The column was then washed with 10 column volumes of Lysis buffer, 10 column volumes of wash buffer, and then eluted with elution buffer at 0.8 ml/min. The eluted peak of A280 was automatically collected.

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NMR Spectroscopy:

Data Collection:
Data Processing: