YWHAB

PDB:2BQ0

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:gi:4507949
Entry Clone Source:MGC
SGC Clone Accession:
Tag:C-terminal hexahistidine tag + TEV cleavage site: egenlyfqslehhhhhh
Host:BL-21(DE3) R3 (phage resistant strain)

Construct

Prelude:
Sequence:

Vector:pET21-TvHR2

Growth

Medium:
Antibiotics:
Procedure:A frozen glycerol stock of transformed E. coli cells was used to inoculate 1 litre of TB plus 100 mg/ml ampicillin. When OD600 reached ~0.5 the temperature was shifted down from 37oC to 25oC 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.

Purification

Procedure
Column 1: Low pressure chromatography using Bio-Rad Econo column (2.5 cm x 13 cm).

Ni-NTA column purification. Buffers: Wash buffer I (WB1): 50 mM Hepes pH 8.0, 300 mM NaCl, 5 % Glycerol, 10 mM Imidazole pH 8.0. Wash Buffer II (WBII): 50 mM Hepes pH 8.0, 300mM NaCl, 30 mM Imidazole pH 8.0. Elution buffer (EB): 50 mM Hepes pH 8.0, 300 mM NaCl, 5 % Glycerol, 250 mM Imidazole pH 8.0.

Procedure: Total volume of Ni-NTA added to BioRad drip column: 4 mls (50%). Resin washed with 12.5 ml of WB1. The supernatant was applied to a column using 5 ml pipette and allowed to pass over the resin. The flow through was collected in a 50 ml falcon tube and applied once more to the column. Two wash steps followed. Wash with 12.5 ml of WBI. Wash with 12.5 ml column vols of WBII. Elute with 14 mls of EB into 7x2 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 hexa-histidine-tagged construct, was over-expressed and purified in-house to a final concentration of 2.5 mg/mL.

Extraction

Procedure
All extraction steps were carried out at 4 ºC.

Extraction buffer (EX): 50 mM Hepes pH 8.0, 300 mM NaCl, 5 % Glycerol, 10 mM Imidazole pH 8.0. 1 tablet protein inhibitor in 10ml EX buffer was added to the 1L growth pellet. Total vol: 45 mL (estimate). Cell breakage: 5 passes through the Emulsiflex C5 high pressure homogeniser. Total vol: 50 mls (estimate). Centrifuge for 30 mins at 16000 rpm and 4°C to remove cell debris. Discard pellet.
Concentration:
Ligand
MassSpec:
Crystallization: The TEV protease cleaved YWHAB was concentrated to 28 mg/mL and distributed into 18x50 mL aliquots before being frozen at -80°C. Crystals grew from a 1:2 ratio mix of protein-to-reservoir (0.05 M magnesium chloride, 0.1 M HEPES pH 7.5, 30 %v/v polyethylene glycol MME 550)
NMR Spectroscopy:
Data Collection:
Data Processing: