YWHAB-ExoS

PDB:2C23

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: enlyfqslehhhhhh
Host:BL-21(DE3)R3 (Phage resistant strain)

Construct

Prelude:
Sequence:

MTMDKSELVQKAKLAEQAERYDDMAAAMKAVTEQGHELSNEERNLLSVAYKNVVGARRSSWRVISSIEQKTERNEKKQQMGKEYREK
IEAELQDICNDVLELLDKYLIPNATQPESKVFYLKMKGDYFRYLSEVASGDNKQTTVSNSQQAYQEAFEISKKEMQPTHPIRLGLAL
NFSVFYYEILNSPEKACSLAKTAFDEAIAELDTLNEESYKDSTLIMQLLRDNLTLWTSENQGDEGENLYFQ

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 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 further 4 hours before harvest.

Purification

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

Buffers: Ni-NTA column purification - 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, 5 % Glycerol, 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 supernatent 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 hexahistidine-tagged construct, was over-expressed and purified in-house to a final concentration of 2.5 mg/ml.

Enzymatic treatment : Add 20 ml of the TEV protease was added to each fraction and left at 4°C overnight.

The following steps were carried out to remove the cleaved products and TEV protease: Change buffer from Elution Buffer to 50 mM Tris pH 8, 150 mM NaCl using a 10-kD cutoff concentrator. Place 200 µl of 50 % Ni-NTA agarose in a 1.5 ml eppendorf tubes, add 1ml of 50 mM Tris pH 8, 150 mM NaCl mix, spin down and remove buffer. Repeat this resin wash step once. Add the TEV treated protein sample to the resin and mix for 30 min. Finally spin down resin and collect the supernatant which contains the cleaved YWHABA.

Extraction

Procedure
All extraction steps were carried out at 4°C.
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 mls (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: Column 1 : Low pressure chromatography using Bio-Rad Econo column (2.5 cm x 13 cm).

Buffers: Ni-NTA column purification - 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, 5 % Glycerol, 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 supernatent 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 hexahistidine-tagged construct, was over-expressed and purified in-house to a final concentration of 2.5 mg/ml.

Enzymatic treatment : Add 20 ml of the TEV protease was added to each fraction and left at 4°C overnight.

The following steps were carried out to remove the cleaved products and TEV protease: Change buffer from Elution Buffer to 50 mM Tris pH 8, 150 mM NaCl using a 10-kD cutoff concentrator. Place 200 µl of 50 % Ni-NTA agarose in a 1.5 ml eppendorf tubes, add 1ml of 50 mM Tris pH 8, 150 mM NaCl mix, spin down and remove buffer. Repeat this resin wash step once. Add the TEV treated protein sample to the resin and mix for 30 min. Finally spin down resin and collect the supernatant which contains the cleaved YWHABA.

NMR Spectroscopy:

Data Collection:

Data Processing: