YWHAQ

PDB:2BTP

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:YWHAQA-s001
Entry Clone Source: Origene
SGC Clone Accession:
Tag:N-terminal hexahistidine tag with integrated TEV protease cleavage site: mhhhhhhssgvdlgtenlyfq*s(m).
Host:E. coli BL21(DE3)-R3

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfqsmMEKTELIQKAKLAEQAERYDDMATCMKAVTEQGAELSNEERNLLSVAYKNVVGGRRSAWRVISS
IEQKTDTSDKKLQLIKDYREKVESELRSICTTVLELLDKYLIANATNPESKVFYLKMKGDYFRYLAEVACGDDRKQTIDNSQGAYQE
AFDISKKEMQPTHPIRLGLALNFSVFYYEILNNPELACTLAKTAFDEAIAELDTLNEDSYKDSTLIMQLLRDNLTLWTSDSAG

Vector:pNIC-Bsa4

Growth

Medium:
Antibiotics:
Procedure: Medium: TB + 50 µg/ml Kanamycin.

1 litre TB in 2.5-L baffled flasks was inoculated with 10 ml overnight culture. The culture was grown at 37°C and transferred to 25°C. When the OD600 reached a value of 3, 1 mM IPTG was added. The final OD600 after an overnight induction was 8.6. The cells were then collected by centrifugation and frozen at -80°C.

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/minute on an AKTA-express system (GE/Amersham). The column was then washed with 10 volumes of Lysis buffer, 10 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.

Concentration : A total of 2 ml of pooled fractions containing YWHAQA were concentrated using a 3K-cutoff vivaspin column. The final concentration was determined from the absorbance at 280 nm using the extinction coefficient was 30 mg/ml.

Extraction

Procedure
Lysis buffer: 50 mM potassium phosphate buffer, pH 7.5, 500 mM NaCl, 10 mM imidazole, 0.5 mM TCEP, Complete® protease inhibitors (1 tablet/50 ml). Frozen cell pellets were thawed on ice over night and resuspended in a total volume of 50 ml lysis buffer. The cells were disrupted by high pressure (20k psi) followed by sonication. Nucleic acids and cell debris were removed by adding 0.15% PEI from a 5% (w/v) stock, stirring for 15 minutes, then centrifugation for 20 minutes at 40000 x g. The supernatant was then further clarified by filtration (0.45 µm).
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/minute on an AKTA-express system (GE/Amersham). The column was then washed with 10 volumes of Lysis buffer, 10 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.

Concentration : A total of 2 ml of pooled fractions containing YWHAQA were concentrated using a 3K-cutoff vivaspin column. The final concentration was determined from the absorbance at 280 nm using the extinction coefficient was 30 mg/ml.
NMR Spectroscopy:

Data Collection:
Data Processing: