TPH2

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Revision

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Entry Clone Accession:IMAGE:40083878
Entry Clone Source: SourceBioScience
SGC Clone Accession:
Tag:N-terminal, TEV protease cleavable hexahistidine tag
Host:

Construct

Prelude:
Sequence:

MHHHHHHSSGVDLGTENLYFQSMLEDVPWFPRKISELDKCSHRVLMYGSELDADHPGFKDNVYRQRRKYFVDVAMGYKYGQPIPRVE
YTEEETKTWGVVFRELSKLYPTHACREYLKNFPLLTKYCGYREDNVPQLEDVSMFLKERSGFTVRPVAGYLSPRDFLAGLAYRVFHC
TQYIRHGSDPLYTPEPDTCHELLGHVPLLADPKFAQFSQEIGLASLGASDEDVQKLATCYFFTIEFGLCKQEGQLRAYGAGLLSSIG
ELKHALSDKACVKAFDPKTTCLQECLITTFQEAYFVSESFEEAKEKMRDFAKSITRPFSVYFNPYTQSIEILKDTRSIENVVQDLRS
DLNTVCDALNKMNQYLGI

MHHHHHHSSGVDLGTENLYFQ*SM is the purification tag plus TEV protease recognition site *.
Vector:pNIC28-Bsa4

Growth

Medium:
Antibiotics:
Procedure:

Purification

Buffers
Procedure

Extraction

Buffers
Procedure
Expression strain
BL21(DE3)-R3-pRARE2
A single colony was used to inoculate 50ml of TB media containing 50 ug/ml kanamycin and 50 ug/ml chloroamphenicol. The starter culture was incubated at 37 °C, 180 rpm and left to grow overnight. The following day the starter culture was used to inoculate 6L of TB media (7 ml starter culture per 1 L) containing with 50 ug/ml kanamycin and grown at 37 °C, 180rpm.When the OD600 reached approximately 0.8 the temperature was reduced to 18°C and after a further 45 minutes the cells were induced by the addition of 0.1 mM IPTG. Expression was continued overnight.

Cell harvest
Cells 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.

Buffers Used:
Binding/Lysis Buffer: 50 mM Hepes (pH 7.5), 500 mM NaCl, 5% Glycerol, 20 mM Imidazole pH 7.5, 0.5 mM TCEP
Wash Buffer: 50 mM Hepes (pH 7.5), 500 mM NaCl, 5% Glycerol, 40 mM Imidazole pH 7.5, 0.5mM TCEP
Elution Buffer: 50 mM Hepes (pH 7.5), 500 mM NaCl, 5% Glycerol, 250 mM Imidazole pH 7.5, 0.5mM TCEP
Gel Filtration Buffer: 50 mM Hepes (pH 7.5), 500 mM NaCl, 5% Glycerol, 0.5mM TCEP
Anion Exchange Buffer A: 50 mM Hepes (pH 7.5), 5% Glycerol, 0.5mM TCEP
Anion Exchange Buffer B: 50 mM Hepes (pH 7.5), 2 M NaCl, 5% Glycerol, 0.5mM TCEP

Cell Lysis
Cell pellets (85g) were dissolved in approximately 240 ml lysis buffer containg EDTA-free protease inhibitors (diluted 1:1000) and lysed by passing through the constant cell disruptor at a constant pressure of 15KPa.The cell debris was pelleted at 35 000 g and the supernatant used for further purification.

Column 1
Ni-NTA (2 mL volume in a gravity-flow column).
The clarified cell extract was incubated with 4 mL pre-equilibriated 50% Ni-NTA resin suspension for 1 hour at 4°C with rotation. The suspension was centrifuged at 900g for 10 min. The supernatant was poured away and the beads were resuspended in lysis buffer and transferred onto a gravity column. The column was then washed with 20ml Binding Buffer (2 x 10ml) and 20 ml Wash Buffer (2 x10 ml). The protein was eluted with 20 ml of elution Buffer (5 x 4 ml fractions).

Column 2
Superdex s200 16/60 Gel Filtration.
Elution fractions (1-3) containing the target protein were applied to the size exclusion chromatography column, s200 (pre-equilibriated in GF buffer) at 1.0 ml/min. 1.0 ml fractions were collected.

Column 3
Pooled fractions from gel filtration were diluted 10 fold with anion exchange buffer A (50mM HEPES, pH 7.5, 5% glycerol, 0.5mM TCEP) and loaded onto 5 mL HiTrapQ column pre-equilibrated with anion exchange buffer A. The protein eluted in a linear gradient from 0 to 1M NaCl, using anion exchange buffer B.

Concentration: To set up crystallization plates the sample was concentrated to 11.6 mg/ml using a 10 kDa mwco concentrator.
Ligand
MassSpec: The mass was analysed by an electrospray time of flight instrument (MSD-TOF)Expected mass: 42116.9 Da Measured mass: 42116.7 Da
Crystallization: Crystals were grown by vapour diffusion in sitting drop at 20°C. A sitting drop consisting of 100 nl protein pre-incubated with 5 mM DL-Tryptophan and 50 nl well solution was equilibrated against a well solution containing 0.20M sodium acetate, 0.1M BisTris-Propane pH 6.5, 20.0% PEG 3350 and 10.0% ethylene glycol
NMR Spectroscopy:
Data Collection: Resolution: 2.63 Å X-ray source: Diamond Light Source beamline IO3
Data Processing: