PTPN4

PDB:2VPH

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:IMAGE:3853914
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal, TEV cleavable hexahistidine tag
Host:BL21(DE3)-R3-pRARE2 (previously known as Rosetta)

Construct

Prelude:
Sequence:

smDNLVLIRMKPDENGRFGFNVKGGYDQKMPVIVSRVAPGTPADLCVPRLNEGDQVVLINGRDIAEHTHDQVVLFIKASCERHSGEL
MLLVRPNAVESTV

Vector: pNIC28-Bsa4

Growth

Medium:
Antibiotics:
Procedure: Transformation: The construct DNA was transformed into homemade chemically competent cells of the expression strain by a standard heat shock procedure.Glycerol stock preparation: A number of colonies from the transformation were used to innoculate 1 ml of LB media containing 50 µg/ml kanamycin and 34 µg/ml chloramphenicol, which was placed in a 37°C shaker overnight. The next day glycerol stocks were prepared from this overnight culture.Expression: A glycerol stock was used to innoculate 50 ml of TB media containing 50 µg/ml kanamycin and 34 µg/ml chloramphenicol, which was placed in a 37°C shaker overnight. The next day this starter culture was used to innoculate 2x 1L of TB media (18 ml starter culture into each) containing 50 µg/ml kanamycin. After 4.5 hours the temperature was reduced to 20°C. After a further 1.5 hours the cells were induced by the addition of 1.0 mM IPTG. The expression was continued overnight.Cell harvest: Cells were spun at 6238x g for 10 mins at 4°C. The cells were resuspended in 50 ml of Lysis Buffer with the addition of 0.2 mM PMSF. The resuspended cell pellet was placed in a -80°C freezer. Lysis Buffer: 50 mM Hepes pH 7.4, 500 mM NaCl, 5% Glycerol, 5 mM Imidazole pH 7.4, 0.5 mM TCEP.Cell Lysis: The resuspended cell pellet was lysed by sonication. PEI (polyethyleneimine) was added to a final concentration of 0.25 % and the cell debris and precipitated DNA were spun down (18000 rpm, JA18 rotor, 90 min).

Purification

Procedure
Column 1: Ni-NTA (2 ml volume in a gravity-flow column).Buffers: Binding Buffer: 50 mM Hepes pH 7.4, 500 mM NaCl, 5% Glycerol, 5 mM Imidazole pH 7.4, 0.5 mM TCEP; Wash Buffer: 50 mM Hepes pH 7.4, 500 mM NaCl, 5% Glycerol, 25 mM Imidazole pH 7.4, 0.5 mM TCEP; Elution Buffer: 50 mM Hepes pH 7.4, 500 mM NaCl, 5% Glycerol, 250 mM Imidazole pH 7.4, 0.5 mM TCEP.Procedure: The clarified cell extract was passed through the column. The column was then washed with Binding Buffer (25 ml) and Wash Buffer (25 ml). The protein was eluted with 10 ml of Elution Buffer.TEV protease digestion: The PTPN4A protein in the elute fraction was exchanged into storage buffer by concentration and dilution, and digested over 48 hours with TEV protease at 4°C.Storage Buffer: 50 mM Hepes pH 7.4, 150 mM NaCl, 0.5 mM TCEPRebinding of impurities to Ni-NTA: The protein was passed through a gravity-flow column containing Ni-NTA resin (2 ml resin volume, pre-equilibrated into Storage Buffer).

Extraction

Procedure
Transformation: The construct DNA was transformed into homemade chemically competent cells of the expression strain by a standard heat shock procedure.Glycerol stock preparation: A number of colonies from the transformation were used to innoculate 1 ml of LB media containing 50 µg/ml kanamycin and 34 µg/ml chloramphenicol, which was placed in a 37°C shaker overnight. The next day glycerol stocks were prepared from this overnight culture.Expression: A glycerol stock was used to innoculate 50 ml of TB media containing 50 µg/ml kanamycin and 34 µg/ml chloramphenicol, which was placed in a 37°C shaker overnight. The next day this starter culture was used to innoculate 2x 1L of TB media (18 ml starter culture into each) containing 50 µg/ml kanamycin. After 4.5 hours the temperature was reduced to 20°C. After a further 1.5 hours the cells were induced by the addition of 1.0 mM IPTG. The expression was continued overnight.Cell harvest: Cells were spun at 6238x g for 10 mins at 4°C. The cells were resuspended in 50 ml of Lysis Buffer with the addition of 0.2 mM PMSF. The resuspended cell pellet was placed in a -80°C freezer. Lysis Buffer: 50 mM Hepes pH 7.4, 500 mM NaCl, 5% Glycerol, 5 mM Imidazole pH 7.4, 0.5 mM TCEP.Cell Lysis: The resuspended cell pellet was lysed by sonication. PEI (polyethyleneimine) was added to a final concentration of 0.25 % and the cell debris and precipitated DNA were spun down (18000 rpm, JA18 rotor, 90 min).
Concentration: The TEV protease cleaved PTPN4A PDZ domain was concentrated to 7.9 mg/ml (measured by 280nm absorbance), distributed into aliquots and frozen at -80°C.
Ligand
MassSpec: Measured: 11026; Expected: 11027.
Crystallization: Crystals grew from a 1:1 ratio of protein to precipitant solution (20 % PEG 3350, 0.2 M (NH4)2H(citrate)), using the vapour diffusion method.
NMR Spectroscopy:
Data Collection: Crystals were cryo-protected by equilibration into precipitant solution containing 20% PEG400, and then flash frozen in liquid nitrogen. Data was collected at the Swiss Light Source, beamline X10.
Data Processing: