ITPK1: Human inositol 1,3,4-triphosphate 5/6 kinase

PDB:2ODT

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:BC018192
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal hexahistidine tag with integrated TEV protease cleavage site: mhhhhhhssgvdlgtenlyfq*s(m).
Host:BL21(DE3)

Construct

Prelude:
Sequence:

```
mhhhhhhssgvdlgtenlyfqsMQTFLKGKRVGYWLSEKKIKKLNFQAFAELCRKRGMEVVQLNLSRPIEEQGPLDVIIHKLTDVIL
EADQNDSQSLELVHRFQEYIDAHPETIVLDPLPAIRTLLDRSKSYELIRKIEAYMEDDRICSPPFMELTSLCGDDTMRLLEKNGLTF
PFICKTRVAHGTNSHEMAIVFNQEGLNAIQPPCVVQNFINHNAVLYKVFVVGESYTVVQRPSLKNFSAGTSDRESIFFNSHNVSKPE
SSSVLTELDKIEGVFERPSDEVIRELSRALRQALGVSLFGIDIIINNQTGQHAVIDINAFPGYEGVSEFFTDLLNHIATVLQGQSTA
M
```

Vector:pNIC-Bsa4

Growth

Medium:
Antibiotics:
Procedure: Cells from glycerol stocks were used to inoculate 100 mL of SOB + Kan (50 µg/mL). The cultures were shaken at 30 °C overnight. The overnight culture was used to inoculate 12 x 750 mL minimal media + Kan (50 µg/mL) (inoculation diluted 1/100). 4 hours later, OD 600 = 0.6, temperature is set to 18°C and an amino acid mix (including selenomethionine) is added. Following 2 hour incubation, target expression was induced by addition of 0.5 mM IPTG. Expression performed at 18°C overnight. Cells were harvested by centrifugation in a SLC-6000 rotor for 10 minutes at 5000 rpm (OD600 3.1; WCW 44 g). Pellets were suspended in 90 mL 50 mM Hepes pH 7.5, 10 % glycerol, 0.5 mM TCEP and 500 mM NaCl, 10mM imidazole and Complete EDTA-free protease inhibitor (Roche Biosciences). Suspended cells were stored at -80°C until further use. Before lysis, 4 µl of 250U/µl benzonase (Novagen) was added to the suspended cells.

Purification

Procedure

Extraction

Procedure
The sample was sonicated (Sonics VibraCell) at 80% amplitude for 3 min (effective time, pulse: 4Â□ on 4Â□ off). The sample was spun for 30 min at 20500 rpm in a Sorvall SA-800 rotor and the soluble fraction decanted and filtered through a 0.45 µm flask filter
Concentration:
Ligand
MassSpec:
Crystallization:ITPK1 crystals were grown by hanging drop vapor diffusion at 293° K. ITPK1 (7 mg/mL) in 20 mM Hepes pH 7.5, 300 mM NaCl, 10 % glycerol, 2 mM TCEP was mixed with an equal amount (0.6 ml) of reservoir solution (100 mM Tris pH 7.8, 1.6 M Citrate). Crystals grew as thin plates diffracting to 2.0 Å on a synchrotron beamline.
NMR Spectroscopy:
Data Collection:A flash-frozen crystal of ITPK1 was used to collect a single wavelength anomalous dispersion dataset to 2.0 Å resolution on the K-edge of selenium at the PSF-beam line (BESSY, Berlin, Germany). Space group was P21 (61.33, 39.55, 67.390 Å; ?=116.7°). XDS/XSCALE was used to process the data and XPREP/SHELXD/SHARP/PIRATE was used to solve the structure. A dataset for refinement was collected later on the ID14-1 beamline at the ESRF (Grenoble, France) to 2.0 Å resolution.
Data Processing: