TBC1D22A

PDB:2QFZ

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:AT17-G1
Entry Clone Source:MGC
SGC Clone Accession:HPC057-F11
Tag:mhhhhhhssgrenlyfqg
Host:E.coli BL21 (DE3) codon plus RIL

Construct

Prelude:
Sequence:

mhhhhhhssgrenlyfqgSEREASRLDKFKQLLAGPNTDLEELRRLSWSGIPKPVRPMTWKLLSGYLPANVDRRPATLQRKQKEYFA
FIEHYYDSRNDEVHQDTYRQIHIDIPRMSPEALILQPKVTEIFERILFIWAIRHPASGYVQGINDLVTPFFVVFICEYIEAEEVDTV
DVSGVPAEVLCNIEADTYWCMSKLLDGIQDNYTFAQPGIQMKVKMLEELVSRIDEQVHRHLDQHEVRYLQFAFRWMNNLLMREVPLR
CTIRLWDTYQSEPDGFSHFHLYVCAAFLVRWRKEILEEKDFQELLLFLQNLPTAHWDDEDISLLLAEAYRLKFAFADAPNHYKK

Vector:pET28-mhl

Growth

Medium: Terrific Broth
Antibiotics:
Procedure:LEX Bubbling

Purification

Procedure
The lysate was centrifuged at 27,000 x g for 60 min and the supernatant was collected and loaded onto a 5 mL HiTrap Chelating HP column (GE Healthcare) loaded with Ni2+, equilibrated with the same binding buffer at 4 ºC. The HiTrap column was washed with 25 mL washing buffer (20 mM HEPES pH 7.5, 300 mM NaCl, 1 mM BME, 30 mM imidazole) and the protein was eluted with a linear concentration gradient of imidazole from 30 mM to 500 mM in the HEPES binding buffer in 50 mL. The fractions containing the target protein were pooled and further purified and desalted using a gel filtration column, Superdex 75 (16/60, GE Healthcare), which was pre-equilibrated with low salt buffer (20 mM HEPES, pH 7.5, 150 mM NaCl, 5 mM DTT). Collected fractions were concentrated using an Amicon Ultra-15 centrifugal filter (5,000 m.w. cut-off) to a

final concentration of 24 mg/mL. Protein concentrations were measured using Bradford assay with purity >95% based on SDS-PAGE analysis.

Extraction

Procedure
The thawed cell pellet (from 1.8 L culture) was resuspended in 100 mL of binding buffer (20 mM HEPES pH 7.5, 300 mM NaCl, 1 mM mercaptoethanol, BME) supplemented with a protease inhibitor cocktail (0.1 mM M benzamidine-HCl and 0.1 mM phenylmethyl sulfonyl fluoride final concentrations), and 0.5% CHAPS. The cells were lysed by liquid fluidizer.
Concentration:24 mg/mL
Ligand
MassSpec:41105.56
Crystallization: Crystallization trials were set up using the sitting drop vapor diffusion method. The protein drop was equilibrated against a reservoir solution (1:1 volume ratio) containing 28.0% PEG400, 0.2M CaCl2, 0.1M NaHEPES pH 7.5. Crystals reached a size of about 30 microns within two to three days.
NMR Spectroscopy:
Data Collection:
Data Processing: