TBC1D14

PDB:2QQ8

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:AT71-E11
Entry Clone Source:MGC
SGC Clone Accession:HPC057-G06
Tag:mhhhhhhssgrenlyfq*g
Host:BL21-CodonPlus(DE3)-RIL

Construct

Prelude:
Sequence:

mhhhhhhssgrenlyfqgNAVLTWNNEILPNWETMWCSRKVRDLWWQGIPPSVRGKVWSLAIGNELNITHELFDICLARAKERWRSL
STGGSEVENEDAGFSAADREASLELIKLDISRTFPNLCIFQQGGPYHDMLHSILGAYTCYRPDVGYVQGMSFIAAVLILNLDTADAF
IAFSNLLNKPCQMAFFRVDHGLMLTYFAAFEVFFEENLPKLFAHFKKNNLTPDIYLIDWIFTLYSKSLPLDLACRIWDVFCRDGEEF
LFRTALGILKLFEDILTKMDFIHMAQFLTRLPEDLPAEELFASIATIQMQSRNKKWAQVLTALQKDSREMEKG

Vector:pET28-mhl

Growth

Medium: Terrific BrothFor selenomethionine (SeMet) labeling, prepackaged M9 SeMET growth media kit (Medicilon) was used following manufacturer instructions
Antibiotics:
Procedure:LEX Bubbling

Purification

Procedure
Column 1: 5 mL HiTrap Chelating HP column (GE Healthcare)
Column 1: Superdex 75 (16/60, GE Healthcare)

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 extraction buffer at 4 degC. The HiTrap column was washed with 25 mL purification buffer and the protein was eluted with a linear concentration gradient of imidazole from 30 mM to 500 mM in the HEPES extraction 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. Collected fractions were concentrated using an Amicon Ultra-15 centrifugal filter (5,000 m.w. cut-off) to a final concentration of 15.1 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 extraction buffer 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:15.1 mg/mL
Ligand
MassSpec: Measured: 38598.31
Expected 38,597.38
SeMet labeled: measured 39073.22
Crystallization:SD12 optimization: 2.0M NaCl, 0.1M Na2HPO4, 0.1M Mes pH 6.5, 0.1M KH2PO4
Buffer 20 mM HEPES pH 8.0, 150 mM NaCl, 5mM DTTSitting drop vaporization
NMR Spectroscopy:
Data Collection:
Data Processing: