L3MBTL2

PDB:3CEY

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:NP_113676
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal: His-tag with integrated TEV protease site: MHHHHHHSSGRENLYFQ*G
Host:*E.coli* BL21 (DE3) codon plus RIL (Stratagen).

Construct

Prelude:
Sequence:

mhhhhhhssgrenlyfqgTGQDALVLGFDWGKFLKDHSYKAAPVSCFKHVPLYDQWEDVMKGMKVEVLNSDAVLPSRVYWIASVIQT
AGYRVLLRYEGFENDASHDFWCNLGTVDVHPIGWCAINSKILVPPRTIHAKFTDWKGYLMKRLVGSRTLPVDFHIKMVESMKYPFRQ
GMRLEVVDKSQVSRTRMAVVDTVIGGRLRLLYEDGDSDDDFWCHMWSPLIHPVGWSRRVGHGIKMSERRSDMAHHPTFRKIYCDAVP
YLFKKVRAVYTEGGWFEEGMKLEAIDPLNLGNICVATVCKVLLDGYLMICVDGGPSTDGLDWFCYHASSHAIFPATFCQKNDIELTP
PKGYEAQTFNWENYLEKTKSKAAPSRLFNMDCPNHGFKVGMKLEAVDLMEPRLICVATVKRVVHRLLSIHFDGWDSEYDQWVDCESP
DIYPVGWCELTGYQLQPPVAAEPATPLKAKEATKKKKKQ

Vector:pET28-MHL

Growth

Medium:TB
Antibiotics:
Procedure:L3MBTL2 protein was expressed in E.coli BL21 (DE3) codon plus RIL in Terrific Broth (TB) in the presence of 50 µg/ml of kanamycin. Cells were grown at 37 degC to an OD600 of 1.5 and induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 1 mM, and incubated overnight at 15 degC.

Purification

Procedure
Column 1: Affinity purification, Ni-NTA
Column 2: Gel filtration, HiLoad 26/60 Superdex 200

The supernatant was incubated with 6mL of 50% slurry Ni-NTA beads by rocking. After 1 hour incubation at 4 ºC, the beads were washed with 50 mL of lysis buffer. The protein was eluted using ~20mL EB.

The eluent from Ni column was loaded onto the gel filtration column in GF buffer at 2.5 mL/min, fraction size 4mL. The fractions containing protein were identified on a SDS-PAGE gel.

Extraction

Procedure
Cells were harvested by centrifugation and pellets were stored in -80 ºC. Prior to purification, the cell pellet was resuspended in lysis buffer. Cells were disrupted by sonication (10 minutes twice) and samples were centrifuged for 60 min at 70000 g. The soluble fraction was subjected to further purification by affinity and size exclusion chromatography.
Concentration:10.5 mg/ml
Ligand
MassSpec:
Crystallization:100mM NaAc 5.3, 100mM NaAC, 15% PEG 3350
NMR Spectroscopy:
Data Collection:
Data Processing: