

L3MBTL2

PDB:3DBB

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:

mhhhhhssgrenlyfqgTGQDALVLGFDWGKFLKDHSYKAAPVSCFKHVPLYDQWEDVMKGMKVEVLNSDAVLPSRVYWIASVIQT
AGYRVLLRYEGFENDASHDFWCNLGTVDVHPIGWCAINSKILVPPRTIHAKFTDWKGYLMKRLVGSRTL PVDFHIKMVESMKYPFRQ
GMRLEVVDKSQVSRTRMAVVDTVIGGRLRLLYEDGSDDDFWCHMWSP LIHPVGWSRRVGHGIKMSERRSDMAHHPTFRKIYCDVP
YLFKKVRAVYTEGGWFEEGMKLEAIDPLNLGNICVATVCKVLLDGYLMICVDGGPSTDGLDWFCYHASSHAIFPATFCQKNDIELTP
PKG YE AQT FNWENYLEKTKSKAAPSR LFNMDCPNHGFKVGMKLEAVDLMEPR LICVATVKRVVHRLLSIHFDGWDSEYDQWVDCESP
DIYPVGWCELTGYQLQPPVAAEPATPLKAKEATK KKKKKQ

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 °C, 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 °C. 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: