L3MBTL

PDB:2RJE

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:NP_056293
Entry Clone Source:
SGC Clone Accession:L3MBTL1_3:A3-APC053
Tag:N-terminal hexahistidine tag with integrated TEV protease cleavage site: mhhhhhhssgrenlyfq*g
Host:E Coli BL21(DE3) RIL CodonPlus

Construct

Prelude:
Sequence:

GEKKECWSWESYLEEQKAITAPVSLFQDSQAVTHNKNGFKLGMKLEGIDPQHPSMYFILTVAEVCGYRLRLHFDGYSECHDFWVNAN
SPDIHPAGWFEKTGHKLQPPKGYKEEEFSWSQYLRSTRAQAAPKHLFVSQSHSPPPLGFQVGMKLEAVDRMNPSLVCVASVTDVVDS
RFLVHFDNWDDTYDYWCDPSSPYIHPVGWCQKQGKPLTPPQDYPDPDNFCWEKYLEETGASAVPTWAFKVRPPHSFLVNMKLEAVDR
RNPALIRVASVEDVEDHRIKIHFDGWSHGYDFWIDADHPDIHPAGWCSKTGHPLQPPLGPRE

Vector:p28a-MHL

Growth

Medium:TB
Antibiotics:
Procedure:A glycerol stock was used to innoclate 20 mL LB media containing 50 µg/mL kanamycin. The culture was grown overnight at 37ºC with shaking. The next day this starter culture was used to innoculate 2L of TB medium which contained 50 µg/mL kanamycin. The culture was grown in LEX at 37ºC to OD600 ~1.1 and was induced with the addition of 0.5 mM IPTG. The temperature was reduced to 18ºC and the culture was incubated for a further 16 hours before harvesting the cells.

Purification

Procedure
Column 1: Affinity purification, open Ni-NTA columnProcedure: The supernatant was incubated with 6mL of 50% slurry Ni-NTA beads by rocking. After 1 hour incubation at 4ºC, the beads were washed with 50 mL of lysis buffer. The protein was eluted using ~20mL EB. Column 2: Gel filtration, HiLoad 26/60 Superdex 200 Prep GradeProcedure: 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 -80ºC. 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:20mg/ml
Ligand
MassSpec:
Crystallization:100mM NaAc 4.6, 100mM NaAC, 5% PEG 4K
NMR Spectroscopy:
Data Collection:
Data Processing: