MBTD1

PDB:3FEO

Revision

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Entry Clone Accession:NP_060113.1
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SGC Clone Accession:MBTD1_5:E5-JMC013
Tag:N-terminal hexahistidine tag
Host:*E. Coli* BL21 (DE3)-V2R-pRARE2

Construct

Prelude:
Sequence:

AKTKAAVSMEGFSWGNYINSNSFIAAPVTCFKHAPMGTCWGDISENVRVEVPNTDCSLPTKVFWIAGIVKLAGYNALLRYEGFENDS
GLDFWCNICGSDIHPVGWCAASGKPLVPPRTIQHKYTNWKAFLVKRLTGAKTLPPDFSQKVSESMQYPFKPCMRVEVVDKRHLCRTR
VAVVESVIGGRLRLVYEESEDRTDDFWCHMHSPLIHHIGWSRSIGHRFKRSDITKKQDGHFDTPPHLFAKVKEVDQSGEWFKEGMKL
EAIDPLNLSTICVATIRKVLADGFLMIGIDGSEAADGSDWFCYHATSPSIFPVGFCEINMIELTPPRGYTKLPFKWFDYLRETGSIA
APVKLFNKDVPNHGFRVGMKLEAVDLMEPRLICVATVTRIIHRLLRIHFDGWEEEYDQWVDCESPDLYPVGWCQLTGYQLQPPASQS
SR

Vector:p28a-MHL

Growth

Medium:TB
Antibiotics:
Procedure:A glycerol stock was used to inoculate 20 mL LB media containing 50 µg/mL kanamycin. The culture was grown overnight at 37°C with shaking. The next day this starter culture was used to inoculate 2L of TB medium which contained 50 µg/mL kanamycin. The culture was grown in LEX at 37°C to OD600 ~3.0 and was induced with the addition of 0.5 mM IPTG. The temperature was reduced to 14°C and the culture was incubated for a further 16 hours before harvesting the cells.

Purification

Procedure
Column 1: Affinity purification: open Ni-NTA Superflow column.
Column 2: Gel filtration: HiLoad 26/60 Superdex 75 Prep
Column 3: Ion exchange: HiTrapQ 5ml.

The supernatant was incubated with 6ml of 50% slurry Ni-NTA beads on ice. After 1 hour incubation the beads were washed with 100ml of lysis buffer. The protein was eluted using 15mL of EB.

The eluent from Ni column was loaded onto the gel filtration column in GF buffer at 1ml/min, fraction size 2mL.

The fractions containing protein were loaded onto the HiTrapQ column containing bufferA at 3ml/min and eluted with gradient of bufferB. 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. The cells were homogenised and sonicated on ice with 10second pulses and 30 second rest intervals. The output level was 8.5 for 6 mins. The sample was centrifuge at 16,000rpm for 1 hour. The supernatant was purified by affinity, size exclusion and ion exchange.
Concentration:10mg/ml.
Ligand
MassSpec:
Crystallization:crystals were obtained by macroseeding in solution containing 20% PEG 3350, 0.2M CaOAc
NMR Spectroscopy:
Data Collection:
Data Processing: