Tb10.70.0370

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Revision

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Entry Clone Accession: Tb10.70.0370
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SGC Clone Accession: Tb10.70.0370:M1-K245:Mac041-A11
Tag:N-terminal tag: mgsshhhhhhssgrenlyfqg
Host:BL21-(DE3)-V2R-pRARE2

Construct

Prelude:
Sequence:

MKRVLLLFDVDGTLTPPRLCQTDEMRALIKRARGAGFCVGTVGGSDFAKQVEQLGRDVLTQFDYVFAENGLLAYRNGLEIHRQSLLN
ALGNDRIVKFVKKTLRLIADLDIPVQRGTFVEYRNGMINVSPIGRNCSQAERDEFEVYDNEHRVRASLIAELENSFPDFGLKYSIGG
QISFDVFPVGWDKTYCLQFVEDDFEEIHFFGDKTQEGGNDYEIYTDKRTIGHKVTSYKDTIAEVEKIIAMK

Vector:pET15-MHL

Growth

Medium:TB
Antibiotics:
Procedure: TbPMM was expressed in *E. coli* BL21-(DE3)-V2R-pRARE2 resistant strain in Terrific Broth (TB) in the presence of ampicillin/chloramphenicol (100 µg/mL and 34 µg/mL respectively). A single colony was inoculated into 100mL of LB with of ampicillin/chloramphenicol (100 µg/mL and 34 µg/mL respectively) in a 250 mL baffled flask and incubated with shaking at 250 rpm overnight at 37 °C. The culture was transferred into 1.0 L of TB with ampicillin/chloramphenicol (100 µg/mL and 34 µg/mL respectively) and 0.15 mL of antifoam (Sigma) in a 1 L bottle and cultured using the LEX system to an OD600 of 5-6, cooled to 15 °C, and induced with 0.5 mM isopropyl-1-thio-D-galactopyranoside (IPTG) overnight at 15 °C.

Purification

Procedure
The cleared lysate was loaded onto a 1.0-2.5 mL Ni-NTA (Qiagen) open column (pre-equilibrated with Binding Buffer) at approximately 1.5-2.0 mL/min. The Ni-NTA column was then washed with 150 mL of Wash Buffer at 2-2.5 mL/min. After washing, the protein was eluted with Elution

Buffer. The eluted sample was applied to a Sephadex S200 16/60 gel filtration column pre-equilibrated with Gelfiltration Buffer on a AKTA explorer system. The fractions corresponding to the eluted protein peak were pooled and further treated with TEV protease overnight to cut the histag. The mixture was loaded onto another 1.0mL Ni-NTA open column and the cut protein was collected from the flow through. Its identity and purity was evalulated by mass spectroscopy and SDS-PAGE gel and then concentrated using a 15 mL Amicon Ultra centrifugal filter device (Millipore). The final concentration of the TbPMM was 10mg/ml and stored at 4 ºC. For long term storage, the protein was flash frozen and stored at -80 ºC.

Extraction

Procedure
The culture was harvested by centrifugation. Pellets from 1 L of culture were resuspended to approximately 40 mL/L of cell culture in Binding Buffer with the addition of protease inhibitors (1 mM benzamidine and 1 mM phenylmethyl sulfonyl fluoride (PMSF)). Resuspended pellets stored at -80 ºC were thawed overnight at 4 °C on the day before purification. Prior to sonication, each pellet from 1 L of culture was pretreated with 0.5 % CHAPS and 500 units of benzonase for 40 minutes at room temperature. After 6 minutes sonication, the cell lysate was centrifuged using a Beckman JA-25.25 rotor at 24,000 rpms for 20 minutes at 4 ºC.
Concentration:
Ligand
MassSpec:
Crystallization: TbPMM was crystallized using hanging drop vapor diffusion. Crystallization buffer is: 2.0M $(NH_4)_2SO_4$, 0.2M NaCl, 0.1M NaCacodylate, pH6.0 at 20 degC.
NMR Spectroscopy:
Data Collection:
Data Processing: