

LmjF32.0080

PDB:3H5Z

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Entry Clone Accession:Q4Q5S8_LEIMA

Entry Clone Source:

SGC Clone Accession:LmjF32.0080:MAC040-B01:C203581

Tag:N-terminal tag: mgsshhhhhssgrenlyfqg

Host:BL21-(DE3)-V2R-pRARE2

Construct

Prelude:

Sequence:

PSNSDAAHAFWSTQVPVQTEDETEKIVFAGPMDEPKTVADIPEEPYPIASTFEWTPNMEAADDIHAIYELLRDNYVEDDDSMFRFN
YSEEFLLQWALCPPNYIPDWHVAVRRKADKKLLAFIAGVPVTLRMGTPKYMKVKAQEKGEGEAAKYDEPRHICEINFLCVHKQLREK
RLAPILIKEATRRVNRNTNVWQAVYTAGVLLPTPYASGQYFHRSLNPEKLVEIRFSGIPAQYQKFQNPMAMLKRNYQLPSAPKNSGLR
EMKPSDVPQVRRILMNYLDSFDVGPVFSDAEISHYLLPRDGVVFTYVVENDKKVTDFFSFYRIPSTVIGNSNYNLLNAAVHYAAT
SIPLHLILDLLIVAHSRGFDVCNMVEILDNRSFVEQLKFGAGDGLRYYFYNNWAYPKIKPSQVALVML

Vector:pET15-MHL

Growth

Medium:TB

Antibiotics:

Procedure:LmNMT 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 26/60 gel filtration column pre-equilibrated with Gelfiltration Buffer on a AKTA explorer system. The fractions corresponding to the eluted protein peak were pooled and its identity and purity was evaluated by mass spectroscopy and SDS-PAGE gel. It was then concentrated using a 15 mL Amicon Ultra centrifugal filter device (Millipore). The final concentration of the LmNMT was 9.2 mg/ml and stored at 4 °C. For long term storage, the protein was flash frozen and stored at -80 °C.

Extraction

Procedure

The culture was harvested by centrifugation. Pellets from 2 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 °C 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 °C.

Concentration:9.2 mg/ml.

Ligand

MassSpec:

Crystallization:The protein was crystallized at 20 degC in LmNMT was crystallized using hanging drop vapor diffusion. Crystallization buffer is: 25.9% PEG1500, 0.2M NaCl, 0.1M NaCacodylate pH5.6, at 20 degC. using the sitting drop vapor diffusion method.

NMR Spectroscopy:

Data Collection:

Data Processing: