

Protein ID (e.g. MH) PP-MetAP

SGC Clone ID construct ID from Polymorph followed by plate ID and plate location)
Tb427.10.15270:MAC04C-E01:C225619

NCBI accession (or equivalent)

Vector pET15-MHL

Construct comments (e.g. mutations, etc.) His tag cut

Construct sequence

AMKTFDFTGPLRPGKITPRRAVPSHIPRPDYADRAGGVSASEEKDRGSKVKVYNIQFLHD
DSKKTAIEIQRITVCQLSREVLDIATAAAKPGITTDDELDRIVHEATVERNMYPSPLNYYGF
PKSVCTSVNEVICHGIPDSRELEEGDILNIDVSSYLNGFHHGDLNETVFIGRPDDDSVRLVH
AAYECLCAGIGVVKPEALYKQVGDAIEACASQYQCSVVRTYTGHGVGHFLFHTSPTVCHY
ANNKSLGMMRPGHVFTIEPMINLGTWQDVTWPKWTSTTKDGRRSAQFEHTMVVTNG
GVEIFTDWVDGVPTYQKQLKEWGIMLPQRKEVGSATAV

Expression Host (e.g. BL21 (DE3)) BL21(DE3)V2RpACYC-LIC+pRARE2

Growth Medium (e.g. TB, LB or M9) TB

Growth procedure Express plasmid in E. coli BL21(DE3)V2RpACYC-LIC+pRARE2 on LB(Luria broth) plate in the presence of carbenicillin(100mg/ml)+chloramphenicol (34 mg/mL). A single colony was inoculated into 25 mL of TB with carbenicillin(100mg/ml)+chloramphenicol (34 mg/mL) in a 50 mL falcon tube and incubated with shaking at 220 rpm overnight at 37 °C. Then the culture was transfer into 1L of TB with carbenicillin(100mg/ml)+chloramphenicol (34 mg/mL), 9ml 0.8M MgSO₄, 180ul *trace element and 0.4 mL of antifoam (Sigma) in a 1 L bottle and cultured using the LEX system to an OD₆₀₀ of ~5, cooled to 15 °C, and induced with 0.5 mM isopropyl-1-thio-D-galactopyranoside (IPTG) overnight at 15 °C

Extraction: Lysis buffer Binding Buffer: 50 mM HEPES pH 7.5, 500 mM NaCl, 5 mM imidazole, and 5 % glycerol

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 -80oC were thawed overnight at 4 °C on the day before purification. Prior to lysis, each pellet from 1 L of culture was pretreated with protease inhibitors, 0.5% CHAPS and 500 units of benzonase. Cells were sonicated for effective time 5 minutes(about 120 watts, pulsed 10s on, 10s off) and the cell lysate was centrifuged using a Beckman JA-25.25 rotor at 24,000 rpms for 30 minutes at 10 °C

Purification buffers Binding Buffer: 50 mM HEPES pH 7.5, 500 mM NaCl, 5 mM imidazole, and 5 % glycerol

Wash Buffer: 50 mM HEPES pH 7.5, 500 mM NaCl, 30 mM imidazole, and 5 % glycerol

Elution Buffer: 50 mM HEPES pH 7.5, 500 mM NaCl, 250 mM imidazole, and 5 % glycerol

Gel Filtration buffer: 10 mM HEPES pH 7.5, 500 mM NaCl, and 5 % glycerol

Purification procedure Affinity column: The cleared lysate was loaded onto a column prepacked with 10 g DE52 (Whatman) anion exchange resin (previously activated with 2.5 M NaCl and equilibrated with Binding Buffer); and subsequently onto a 4 mL Ni-NTA (Qiagen) column pre-equilibrated with Binding Buffer at approximately 1 – 1.5 mL/min. After the lysate was loaded, the DE52 was further washed with 20 mL of Binding Buffer. The Ni-NTA column was then washed with 200 mL of Wash Buffer. After washing, the protein was eluted with 15 mL of Elution Buffer and treated with 1mM TCEP. The protein was then incubated with TEV to remove his-tag and evaluated by mass spectroscopy and SDS-PAGE gel.

Gel filtration: The sample was loaded onto a Sephadex S200 26/60 column equilibrated with Gel Filtration Buffer. The fractions from the peak corresponding to protein were collected.

Crystallization plate, well, drop (e.g. MAY024:A2-1 for plate MAY024, well A2, drop 1) IC-MAZ65K:H10-2

Cryo conditions 20% glycerol

Crystallization procedure (if above conditions are not sufficient) sitting drop; 0.3 μ L 11 mg/mL protein + 0.3 μ L buffer with 2mM MnCl₂+5mM MgCl₂, TCEP

Crystallization 0.1 tris 6.5, 28% peg2k MME

*Trace element "CoCl₂-6H₂O 6.3 mM

MnSO₄-5H₂O/ MnSO₄-H₂O 33.19 mM

CuCl₂-2H₂O 5.86 mM

H₃BO₃ 8.09 mM

Na₂MoO₄-2H₂O 8.27 mM

ZnSO₄-7H₂O 6.95 mM

FeSO₄-7H₂O 107.91 mM

CaCl₂-2H₂O 67.98 mM

AlCl₃-6H₂O 4.14 mM

NiCl₂-6H₂O 8.41 mM