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

SGC Clone ID construct ID from Polymorph followed by plate ID and plate location)
Tb427tmp.160.5560:MAC049-C01:C223709

NCBI accession (or equivalent)

Vector pET15-MHL

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

Construct sequence
MEKGSPSDLNGVDYSVDNPLFALSPLDGRYKRQTKALRAFFSEYGFFRYRVLVEVEYFTA
LCKDVPTIVPLRSVTDEQLQKLRKITLDCFSVSSAEEIKRLERVTNHDIKAVEYFIKERMDT
CGLSHVTEFVHFGLTSQDINNTAIPMMIRDAIVTLYLPALDGIIGSLTSKLVDWDVPMLAR
THGQPASPTNLAKEFVVWIERLREQRRQLCEVPTTGKFGGATGNFNAHLVAYPSVNWRA
FADMFLAKYLGLKRQQATTQIENYDHLAALCDACARLHVILIDMCRDVWQYISMGFFK
QKVKEGEVGSSTMPHKVNPIDFENAEGNLALSNALLNFFASKLPISRLQRDLTDSTVLRN
LGVPIGHACVAFASISQGLEKLMISRETISRELSSNWAVVAEGIQTVLRRECYPKPYETLKK
LTQGNTDVTEEQVRNFINGLTDISDDVRAELLAITPFTYVGYVPRFSAK

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(Lauria 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 ºC. 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 ºC, and induced with 0.5 mM isopropyl-1-thio-D-galactopyranoside (IPTG) overnight at 15 ºC

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 ºC

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 evalulated 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 dimer protein were collected.

Crystallization plate, well, drop (e.g. MAY024:A2-1 for plate MAY024, well A2, drop 1) IC-MAZ5Z5:E7-2

Cryo conditions 15%glycerol

Crystallization procedure (if above conditions are not sufficient) sitting drop; 1 µL 8 mg/mL protein + 1 µL buffer with 5mM AMP+5mM MgCl2,tcep

Crystallization 3.1M NaFormate,0.1M tris PH8

*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