Structure Title (e.g. Human malate dehydrogenase + NAD) Leishmania donovani map kinase (LdBPK_331470) in complex with AMP-PNP

PDB ID 4QNY

Protein ID (e.g. MH) LdBPK_331470

SGC Clone ID construct ID from Polymorph followed by plate ID and plate location) LdBPK_331470.1:M1-E353

NCBI accession (or equivalent) LdBPK_331470

Vector p15-mhl

Construct comments (e.g. mutations, etc.)

Construct sequence
MPATKSLAELQAEVCRLDDRYLLERIIGAGSYGVVIRARDTKSDNRLVAMKRVNKEIFEE
VILAKRILREIKLLAHFNDDNIIGLRNILTPEDPENFDHFYIVMDIMETDLKQVLRSGQELT
EAHIQFFIYQALRALHIIHSAGVIHRDITPANILVNTNCDLKICDFGLAKEENDQGEYMTD
YVTMRWYRAPELVMEDKDYSAQIDVWGIGCILGELLGSRPLFQGKDRVNQLDKIVDVIG
TPSEEDINSVGSSAAQKYLKKKSHRPQADWRQRYPTASPEALDLLRHMLVFNPKRRITVL
QAMRHPFLEQLHDDADDNLSYALFRFDENEQKTIVDVKRAIYEESVKFHNE

Tag MHHHHHHSSGRENLYFQG

Expression Host (e.g. BL21 (DE3))

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

Growth procedure PF10-0086 was expressed in E. coli BL21-(DE3)-Rosetta-Oxford cells in Terrific Broth (TB) in the presence of ampicillin/chloramphenicol (50 microg/mL and 25 microg/mL respectively). A single colony was inoculated into 10 mL of LB with of ampicillin/chloramphenicol (50 microg/mL and 25 microg/mL respectively) in a 50 mL Falcon tube and incubated with shaking at 250 rpm overnight at 37 degC. The culture was transferred into 50 mL of TB with 50 microg/mL ampicillin in a 250 mL shaking flask and incubated at 37 degC for 3 hours. Then the culture was transfered into 1.8 L of TB with 50 microg/mL kanamycin and 0.3 mL of antifoam (Sigma) in a 2 L bottle and cultured using the LEX system to an OD 600 of ~5, cooled to 15 degC, and induced with 0.5 mM isopropyl-1-thio-D-galactopyranoside (IPTG) overnight at 15 degC.

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 4 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 degC were thawed overnight at 4 degC on the day before purification. Prior to mechanical lysis, each pellet from 1 L of culture was pretreated with 0.5 % CHAPS and 500 units of benzonase for 40 minutes at room temperature. Cells were mechanically lysed with a microfluidizer (Microfluidizer Processor, M-110EH) at approximately 18000 psi; and the cell lysate was centrifuged using a Beckman JA-25.50 rotor at ~75000 x g (24000 rpms) for 20 minutes at 10 degC.

Purification buffers

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

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

Purification buffers Gel Filtration Buffer: 10 mM HEPES, pH 7.5, 500 mM NaCl

Purification procedure STEP1: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 3 mL Ni-NTA (Qiagen) column pre-equilibrated with Binding Buffer at approximately 1 – 1.5 mL/min. The volume of the Ni-NTA resin was pre-determined by the predicted protein yield from test expression analysis. 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 at 2 – 2.5 mL/min. After washing, the protein was eluted with 15 mL of Elution Buffer and TCEP was added to 0.5 mM after approximately 15 more minutes.

STEP2:The sample was loaded onto a Sephadex S75 26/60 gel filtration column pre-equilibrated with 10 mM HEPES, pH 7.5 and 500 mM NaCl. The collected fractions corresponding to the correct eluted protein peak were concentrated using a 15 mL Amicon Ultra centrifugal filter device (Millipore). TECEP (5mM) and MgCl2 (5mM) was added to the concentrated protein. The protein sample identity were evalulated by mass spectroscopy. The concentrated sample (6 mg/ml) was stored at 4 degC.

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

Crystallization method (e.g. hanging drop) sitting drop vapor diffusion

Crystallization buffers 17% PEG3350, Tris pH 8.5

Cryo conditions 15% glycerol

Crystallization temperature 293K

Crystallization procedure (if above conditions are not sufficient) 6mg/ml the protein crystalized in 17% PEG3350, Tris pH 8.5, 0.2 M MgCl2, 5% glycerol (hanging drop), freezing cryo, 15% glycerol, 2mM AMPPNP

Ligand (mention where and when added, molar ratio) 2mM AMPPNP added to the protein before setting up the plate