

Structure Title (e.g. Human malate dehydrogenase + NAD) Plasmodium falciparum adenylate kinase (PF10_0086), in complex with ADP

PDB ID 3TLX

Protein ID (e.g. MH) PF10-0086

SGC Clone ID construct ID from Polymorph followed by plate ID and plate location)
PF10_0086:M1-G242

NCBI accession (or equivalent) PF10-0086

Vector p15-mhl

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

Construct sequence

MNENLENFSTIDLLNELKRRYACLSKPDGRYIFLGAPGSGKGTQSLNLKSHCYCHLSTG
DLLREAAEKKTELGLKIKNIINEGKLVDDQMVLVDEKLKTPQCKKGFIILDGYPRNVKQ
AEDLNKLLQKNQTKLDGVFYFNVPDEVLVNRISGRLIHKPSGRIYHKIFNPPKVPFRDDV
TNEPLIQREDDNEDVLKKRLLTVFKSETSPLISYYKNKNLLINLDATQPANDLEKKISQHID
G

Tag MHHHHHHSSGRENLYFQG

Expression Host (e.g. BL21 (DE3)) Ros-Ox

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 transferred 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 rpm) 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). TCEP (5mM) and MgCl₂ (5mM) was added to the concentrated protein. The protein sample identity were evaluated by mass spectroscopy. The concentrated sample (62 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 25% PEG 3350, 0.2 M Ammonium Acetate, 0.1 M Hepes,

Cryo conditions 25% glycerol

Crystallization temperature 293K

Crystallization procedure (if above conditions are not sufficient) 25% PEG 3350, 0.2 M Ammonium Acetate, 0.1 M Hepes, 5 mM ADP, 5 mM MgCl₂, 0.3 mM glyglyglycine, VAPOR DIFFUSION, SITTING DROP, temperature 293K, pH 7.5

Ligand (mention where and when added, molar ratio) 5mM ADP added to the protein before setting up the plate