Target cGMP protein Kinase from plasmodium Vivax (PVX_084705) in complex with AMPPNP

PDB Code 5DZC

Entry Clone Source

Entry clone accession

Construct sequence
MRCNERNKKKAIFSNDDFSGEDTLMEDHLQLREKLSEDIEMIKASLKNNLVCSTLNDNEI
LTLSNYMQFFVFKGGDLVIKQGEKGSYFFIINSGKFDVYVNDKKVKSMGKGSSFGEAALI
HNTQRSATIMAETDGTLWGVQRSTFRATLKQLSNRNFNENRSFIDSVSVFDMLTEAQKN
MITNACVIQMFKPGETIVKQGDYGDVLFILKEGKATVFINDKEIRVLNKGSYFGERALLY
DEPRSATIIAKEPTACASICRKLLNIVLGNLQVVLFRNIMTEALQQSEIFRQFSAEQLNDLA
DTAIVRDYPANYHILHKDKVKSVKYLIVLEGKVELFLDDESIGILTRGKSFGDQYVLNQK
QKFRHTVKSLDVCKIALITESCLADCLGDNNIDASIDHNNKKSIIKKMYIFRYLSEQQCNL
LIEAFRTTRYEEGDYIIQEGEVGSRFYIIKNGEVEVTKNGKRLRTLGKNDYFGERALLYDE
PRTASIISKATSVECWFVDKSVFLQIIQGPMLTHLEERIKMQDTKVEMHELETERIIGRGTF
GTVKLVHHKPTQIRYALKCVSKRSIISLNQQNNIKLEREITAENDHPFIIRLVRTFKDSNCFY
FLTELVTGGELYDAIRKLGLLSKPQAQFYLGSIILAIEYLHERNIVYRDLKPENILLDKQGY
VKLIDFGCAKKIQGRAYTLVGTPHYMAPEVILGKGYGCTVDIWALGVCLYEFICGPLPFG
NDQEDQLEIFRDILTGQLTFPDYVSDQDSINLMKRLLCRLPQGRIGCSINGFKDIKEHAFFG
NFNWDKLAGRLLEPPLVSKGETYAEDIDIKQIEEEDALNEGEPLDGDDSWDVDF

Vector PET-15MHL

Tag N-terminal His-tag with integrated TEV protease site: MHHHHHHSSGRENLYFQG

Host Cell BL21(DE3)-CodonPlus-RIL-pACYC LamP

Growth Protocol PVPKG (PVX_084705) was expressed in E. coli BL21 (DE3) CodonPlus-RIL LamP in Terrific Broth (TB) in the presence of Ampicillin/chloramphenicol (100µg/mL and 34µg/mL respectively). A single colony was inoculated into 100 mL of LB with 100µg/mL Ampicillin and 34 chloramphenicol in a 250 mL shaking flask and incubated at 37 ºC for Overnight. The culture was transferred in 4,0.8L TB with Ampicillin/chloramphenicol and 0.4 mL of antifoam (Sigma) in 1 L bottles and cultured using the LEX system to an OD600 of 4 t0 5. The culture was cooled to 15 ºC, and isopropyl-1-thio-D-galactopyranoside (IPTG) was added to 0.4 mM, and the culture was incubated overnight at 15 ºC.

Extraction Buffer Binding Buffer: 50 mM HEPES pH 7.5, 300 mM NaCl, 5 mM imidazole, and 5 % glycerol

Extraction Method The culture was harvested by centrifugation and the cell pellet was suspended in 200 mL of binding buffer (50 mM HEPES, pH 7.5, 300 mM NaCl, 5% glycerol, and 15 mM imidazole) with protease inhibitor and kept in 50 mL Falcon tubes at – 80 ºC. Before purification, the cell suspension was thawed in the morning before starting purification. Prior to mechanical lysis, each tube of cell suspension was pretreated with 0.5 % NP40 and 500 units of benzonase (per 40 mL of resuspended cell pellet) for 15 minutes at room temperature. Then the cells were mechanically lysed with a microfluidizer (Microfluidizer Processor, M-110EH) at approximately 18000 psi. The lysate was centrifuged at 15500 rpm for 60 minutes at 10 ºC.

Purification Buffer 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

Purification The cleared cell lysate was loaded directly onto a 5 mL Ni-NTA (Qiagen) column. When all the lysate was loaded, the column system was washed with 20 mL binding buffer. The Ni-NTA column was then washed with 200 mL of Wash Buffer (50 mM HEPES pH 7.5, 300 mM NaCl, 30 mM imidazole, and 5 % glycerol). After washing, the protein was eluted from the Ni-NTA column with 15-20 mL of Elution Buffer (50 mM HEPES pH 7.5, 300 mM NaCl, 250 mM imidazole, and 5 % glycerol). TCEP was added to 1 mM.

PVPKG His-tag was cleaved with Tev protease overnight at 4 ºC in the presence of 1 mM TCEP (Tris(2-Carboxyethyl) phosphine Hydrochloride). The cleaved sample was applied to a 3 mL Ni-NTA column pre-equilibrated with 10 mM HEPES, pH 7.5, 300 mM NaCl, and 15 mM imidazole. The cleaved sample applied to the Ni-NTA column. The flow-through was collected; and the column was rinsed was an additional 5 mL of 10 mM HEPES, pH 7.5, 300 mM NaCl, and 15 mM imidazole.

The His tag cleaved sample was then loaded onto a superdex 200 gel filtration column. The eluted protein (in 10 mM Hepes, pH 7.5 and 300 mM NaCl) was concentrated using a 15 mL Amicon Ultra centrifugal filter device (Millipore) with a 5 kDa cutoff. Pf-Chk (PF14-0020) was concentrated to 10 mg/mL and flash frozen in N2(l) and stored at -80C. Protein was diluted to 6.5 mg.mL for crystallization.

Stock Concentration 6.5 mg/ml

Crystallization The protein was crystallized at 20 ºC in 18.2% PEG3350, 0.1 M HEPES pH 7.0, 0.1M Succinate, in sitting drop method. 1mM TECEP, 2mM MgCl2, 5mM AMPPNP added directly to the concentrated protein immediately prior to setting up the crystallization plate.