Pv-EK: Plasmodium vivax ethanolamine kinase

PDB:2QG7

Revision

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Entry Clone Accession: Pv091845
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SGC Clone Accession:PV-PF11_0257:; plate G:H4
Tag:N-terminal His6-tag with integrated TEC cleavage site (*): mgsshhhhhhssglvpr*gs
Host:E. coli BL21-(DE3)-R3-pRARE2

Construct

Prelude:N.B. Wrong sequence in NCBI - use PlasmoDB
Sequence:

gsEQKKRRLEEGTRANSVAESSPPFPLRSKTSVGSTNSQITETKNKQGVYPITESNLRILEGEDRSEKAKELLKKYVSNVFENEKTL
YIYCKYVMLHYGKDLVNPNEVDSLEFQIINGGITNILIKVKDMSKQAKYLIRLYGPKTDEIINRREKKISCILYNKNIAKKIYVFF
TNGRIEEFMDGYALSREDIKNPKFQKLIAKNLKLLHDIKLNENLYKELQVTQKVPGTRPSFLWNTIWKYFHLLNEERKKICSFDAKA
NILKLIDFDVLRDSIVEVESLCKRENSPIVLCHCDLLSSNIINTVGGGEAGELGEAGETGEGGETGEGGETGEGGETGEGGEGDSIS
FIDFEYSCPMERAYDIANHFNEYAGFNCDWDLTPSKEEEYHFIMHYLGTDDEELINQLIREIQPFYICSHINWGLWSLLQGMHSSID
FDFINYGMTRLTASCLPIFRSKV

Vector:p15TV-L

Growth

Medium:TB
Antibiotics:100 microG/mL ampicillin and 34 microG/mL chloramphenicol
Procedure:A single colony was inoculated into 10 mL of LB with of Antibiotics and incubated with shaking at 250 rpm overnight at 37 degC. The culture was transferred into 50 mL of TB with Antibiotics in a 250 mL shaking flask and incubated at 37 degC for 3 hours. The culture was then transferred into 1.8 L of above-specified growth medium with Antibiotics and 0.3 mL of antifoam (Sigma) in a 2L bottle and cultured using the LEX system to an OD600 of ~5, cooled to 15 degC and induced with 0.5 mM isopropyl-1-thio-D-galactopyranoside (IPTG) overnight at 15 ºC.

Purification

Procedure
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 1.0 Â□ 2.5 mL Ni-NTA (Qiagen) column pre-equilibrated with Binding Buffer at approximately 1 Â□ 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 Â 2.5 mL/min. After washing, the protein was eluted with 15 mL of Elution Buffer. TCEP was added to 1 Â 5 mM after approximately 15 more minutes.

The His6-tag was cleaved with thrombin overnight at 4 degC and dialysed into Crystal Buffer. The cleaved sample was loaded onto a Sephadex S200 26/60 column equilibrated with Gel Filtration Buffer. The fractions from the peak corresponding to monomeric protein were pooled and concentrated using a 15 mL Amicon Ultra centrifugal filter device (Millipore). The protein sample identity and purity were evalulated by mass spectroscopy and SDS-PAGE gel. The concentrated protein was flash frozen and stored at -80 degC.

Extraction

Procedure
Cells were resuspended to approximately 40 mL/L of cell culture in Binding Buffer with protease inhibitor (1 mM benzamidine-HCl and 1 mM phenylmethyl sulfonyl fluoride, PMSF). Resuspended pellets stored at -80 degC were thawed overnight at 4 ºC 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 at ~75000 x g for 20 minutes at 10 degC.
Concentration:
Ligand
MassSpec:
Crystallization: The protein was crystallized at 20 degC in 1.75 M Ammonium Sulphate, 100 mM Sodium Citrate, pH 7.0 using the sitting drop vapor diffusion method.
NMR Spectroscopy:
Data Collection:
Data Processing: