Py-1cys-Prx: Plasmodium yoelii 1-cys-peroxidoxin (PY04285 - ortholog of PF08_0131)

PDB:1XCC

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:PY04285
Entry Clone Source: Plasmodium yoelii 17XNL genomic DNA
SGC Clone Accession:PY04285:; plate 2002:B10
Tag:N-terminal: His-tag with integrated TEV protease site: mgsshhhhhhssgrenlyfqghm. C-terminal: gs
Host:BL21 (DE3) Rosetta-2 from Novagen

Construct

Prelude:
Sequence:

mgsshhhhhhssgrenlyfqghmGYHLGATFPNFTAKASGIDGDFELYKYIENSWAILFSHPNDFTPVCTTELAELGKMHEDFLKLN
CKLIGFSCNSKESHDKWIEDIKYYGKLNKWEIPIVCDESRELANKLKIMDEQEKDITGLPLTCRCLFFISPEKKIKATVLYPATTGR
NAHEILRVLKSLQLTYTTPVATPVNWNEGDKCCVIPTLQDDEISKHFKNEITKVEMPSKKKYLRFVNLgs

Vector:p11

Growth

Medium: Studier auto-induction media
Antibiotics:
Procedure:A single colony was inoculated into 10 mL of LB with of carbenicillin (50 microG/mL) and incubated with shaking at 250 rpm overnight at 37 degC. The culture was transferred into 50 mL of above-specified medium with carbenicillin (50 microG/mL) 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 carbenicillin (50 microG/mL)in a 2L baffled flask and grown at 37 degC overnight in an Innova shaker from New Brunswick Scientific (150 rpm).

Purification

Procedure
The cleared lysate was loaded onto a column of 3 mL Ni-NTA from Qiagen at 4 degC. The column was washed with 150 mL Wash Buffer and the protein was eluted with 15 mL Elution Buffer. About 25 mg of pure protein was obtained from 1L of cell culture. The purified protein

was dialyzed overnight into Crystal Buffer at 4 degC and concentrated using a Amicon Ultra centrifugal filter device (15 kD cutoff).

Extraction

Procedure
Cultures were centrifuged and the cell pellets were resuspended in binding buffer with protease inhibitor (1 mM benzamidine-HCl and 1 mM phenylmethyl sulfonyl fluoride, PMSF)and flash frozen. The thawed cell pellet was lysed by a combination of 0.5% CHAPS (Sigma) and sonication (1x 30 sec). Lysate was cleared by centrifugation and passed through DE52 from Whatman in 0.5 M NaCl.
Concentration:15 mg/mL
Ligand
MassSpec:
Crystallization: The protein was crystallized by means by hanging drop vapor diffusion in a VDXm plate. The plate was set with 1.5 microL uncleaved protein (15 mg/mL) and 1.5 microL buffer in each drop, and 350 microL reservoir volume per well. Crystals emerged in 23% Peg 3350, 0.1M Bis-Tris pH 5.5, 0.2M ammonium sulfate and 5% ethylene glycol at 20 degC.
NMR Spectroscopy:
Data Collection:
Data Processing: