

Py-2Cys-Prx

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Revision

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Entry Clone Accession:PY00414

Entry Clone Source:Plasmodium yoelii 17XL

SGC Clone Accession:

Tag:N-terminal: His6-tag with integrated TEV protease site: mgsshhhhhssgrenlyfq*g

Host:E. coli BL21-(DE3)-CodonPlus-RIL from Stratagene

Construct

Prelude:

Sequence:

mgsshhhhhssgrenlyfqgQAPSFKA EAVFGDNTFGEVSLSDFIGKKYVLLYFYPLDFTFVCPSEIIALDKALDSFKERNVELLG
CSVDSKFTHLAWKKTPLSQGGIGNIKHTLISDISKSIARSYDVLFNESVALRAFLIDKQGVVQHLLVNNLALGRSVDEILRLIDAL
QHHEKYGDVCPANWQKGKESMKPSEEGVAKYLSNL

Vector:p15-tev-lic

Growth

Medium:

Antibiotics:

Procedure:Py-2Cys-Prx was expressed in E. coli BL21-(DE3)-CodonPlus-RIL in Terrific Broth (TB) in the presence of kanamycin/chloramphenicol (50 microgram/mL and 25 microgram/mL respectively). A single colony was inoculated into 10 mL of LB with of kanamycin/chloramphenicol (50 microgram/mL and 25 microgram/mL respectively) in a 50 mL Falcon tube and incubated with shaking at 250 rpm overnight at 37 °C. The culture was transferred into 50 mL of TB with 50 microgram/mL kanamycin in a 250 mL shaking flask and incubated at 37 °C for 3 hours. Then the culture was transfer into 1.8 L of TB with 50 microgram/mL kanamycin and 0.3 mL of antifoam (Sigma) in a 2 L bottle and cultured using the LEX system to an OD600 of 10, cooled to 15 °C, and induced with 0.5 mM isopropyl-1-thio-D-galactopyranoside (IPTG) overnight at 15 °C.

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 Å × 2.5 mL Ni-NTA (Qiagen) column pre-equilibrated with Binding Buffer at approximately 1 Å × 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. Each Ni-NTA column was then washed with 200 mL of Wash Buffer (50 mM HEPES pH 7.5, 500 mM NaCl, 30 mM imidazole, and 5 % glycerol) at 2 Å × 2.5 mL/min. After washing, the protein was eluted with 15 mL of Elution Buffer (50 mM HEPES pH 7.5, 500 mM NaCl, 250 mM imidazole, and 5 % glycerol). EDTA was immediately added to the elution fraction to 1 mM; and DTT was added to 1 Å × 5 mM after approximately 15 more minutes. The protein was dialyzed overnight at 4 oC against 10 mM HEPES, pH 7.5, 500 mM NaCl; and finally concentrated using a 15 mL Amicon Ultra centrifugal filter device (Millipore). The concentrated sample was stored at 4 oC.

Extraction

Procedure

The culture was harvested by centrifugation. Pellets from 2 L of culture were resuspended to approximately 40 mL/L of cell culture in Binding Buffer (50 mM HEPES pH 7.5, 500 mM NaCl, 5 mM imidazole, and 5 % glycerol) with the addition of protease inhibitors (1 mM benzamidine and 1 mM phenylmethyl sulfonyl fluoride (PMSF)). Resuspended pellets stored at -80 oC 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 a Beckman JA-25.50 rotor at ~75000 x g (24000 rpms) for 20 minutes at 10 oC.

Concentration: 4.3 mg/mL for Py-2Cys-Prx with His6-tag.

Ligand

MassSpec:

Crystallization: Purified Py-2Cys-Prx was crystallized using the sitting drop vapor diffusion method in a Linbro plate with 300 µL of mother liquor at 18 °C. 1.5 µL of the protein solution was mixed with 1.5 µL of the reservoir solution containing 1.6 M ammonium sulfate, 100 mM HEPES, pH 6.8, 200 mM NaAc, 20 mM NaBr, 5% ethylene glycol. Crystals appeared in 3-5 days.

NMR Spectroscopy:

Data Collection:

Data Processing: