PRDX4

PDB:2PN8

Revision

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Revised by:created
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Entry Clone Accession:gi|5453549
Entry Clone Source:MGC
SGC Clone Accession:PRDX4A-c004
Tag:N-terminal, TEV cleavable hexahistidine tag. Tag sequence: mhhhhhhssgvdlgtenlyfq(*)sm
Host:BL21(DE3)-R3-pRARE2

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfq*smPAPYWEGTAVIDGEFKELKLTDYRGKYLVFFFYPLDFTFVCPTEIIAFGDRLEEFRSINTEVV
ACSVDSQFTHLAWINTPRRQGGLGPIRIPLLSDLTHQISKDYGVYLEDSGHTLRGLFIIDDKGILRQITLNDLPVGRSVDETLRLVQ
AFQYTDKHGEVCPAGWKPGSETIIPDPAGKLKYFDKLN

Vector:pNIC28-Bsa4.

Growth

Medium:TB
Antibiotics:
Procedure:10µl of BL21(DE3)-R3-pRARE2 glycerol stock were inoculated into 60ml of TB with 50µg/ml of kanamycin and 34µg/ml chloramphenicol and grown overnight at 37°C, 200rpm. 10ml of overnight culture were added to 1L of TB with 50µg/ml kanamycin and incubated at 37°C, 160rpm. After the OD600 reached 1.0, the temperature was dropped to 18 °C and 500ul of 1M IPTG was added to the final concentration of ~0.5mM. The culture was then incubated with shaking overnight at 18 °C, 160rpm. The following morning the 4L culture was harvested and centrifuged for 15min at 4000rpm. Supernatant was discarded and cell pellets were resuspended in 80ml of a lysis buffer and frozen at -80 °C.

Purification

Procedure
The cell extract was loaded on the AKTA Express system The extinction at 280nm was monitored and fractions were collected and analyzed by SDS-PAGE. Positive fractions were pooled and concentrated.

Extraction

Procedure
The thawed cells were broken by 5 passes at 16.000 psi through a high pressure homogeniser followed by centrifugation for 45 min at 15.000rpm.
Concentration: Using VivaSpin-15 concentrators with 10kDa cutoff, the sample was concentrated to 8mg/ml. Concentrations were determined from the absorbance at 280nm using NanoDrop.
Ligand
MassSpec: Calculated mass of the construct was 24001. The mass of 24000 was confirmed by the mass spec.
Crystallization: Crystals were grown by vapor diffusion at 20°C in 3ul hanging drops. The drops were prepared by mixing 2ul of protein solution and 1ul of precipitant consisting of 0.1M HEPES pH 7.5, 10% PEG 10K and 8% Ethylene Glycol. Crystals were transferred to a cryo-protectant consisting of 10% Ethylene Glycol and 90% well solution before flash-cooling in liquid nitrogen.
NMR Spectroscopy:
Data Collection:Resolution: 1.8Å; X-ray source: SLS beam X10SA.
Data Processing: