GPX7: Human glutathione peroxidase 7

PDB:2P31

Revision

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Revised by:created
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Entry Clone Accession:BC032788
Entry Clone Source:MGC
SGC Clone Accession:
Tag:TEV-cleavable (*), N-terminal histag.
Host: Rosetta-R3

Construct

Prelude:
Sequence:

```
mhhhhhhssgvdlgtenlyfq*SMQQEQD FYDFKAVNIRGKLVSLEKYRGSVSLVVNV ASECGFTDQHYRALQQLQRDLGPHHFN
VL AFPCNQFGQQEPDSNKEIESFARRTYSVS FPMFSKIAVTGTGAHPAFKYLAQTSGKEP TWNFWKYLVAPDGKVVGAWDPTVS
VEEVR PQITALVR
```

Vector:pNIC28-Bsa4

Growth

Medium:TB + 50 microG/ml Kanamycin + 34 µg/ml chloramp
Antibiotics:
Procedure:3 litre TB in 2.5-L baffled flasks were inoculated with 10 ml overnight culture and grown at 37degC. The protein expression was induced with 1 mM IPTG at OD600 = 4.6 at 18degC overnight. The cells were collected by centrifugation and frozen at -80degC.

Purification

Procedure
Ni-affinity:The cell extract was loaded on the column at 0.8 ml/minute on an AKTA-express system (GE/Amersham). The column was then washed with 10 volumes of lysis buffer, 10 volumes of wash buffer, and then eluted with elution buffer at 0.8 ml/min. The eluted peak of A280 was automatically collected.

Gel filtration: The eluted fractions from the Ni-affinity Histrap columns were loaded on the gel filtration column in GF buffer at 0.80 ml/min. Eluted proteins were collected in 2 ml fractions and analyzed on SDS-PAGE gels.

Concentration: DTT was added to the protein to a final concentration of 5 mM. The protein was concentrated in Amicon (5 K) to 12 mg/ml and stored at -80degC. The protein concentration was determined spectrophotometrically using the predicted molar extinction coefficient (26930 M-1cm-1).

Extraction

Procedure
Frozen cell pellets were thawed at 37°C and resuspended in a total volume of 150 ml lysis buffer. The cells were disrupted by high pressure (20 kpsi) and nucleic acids and cell debris removed by adding 0.15% PEI , followed by centrifugation for 30 minutes at 40,000xg. The supernatant was further clarified by filtration (0.45 μm).
Concentration:
Ligand
MassSpec:
Crystallization: Crystals were grown by vapor diffusion at 20degC. A sitting drop consisting of 75 nanoliter protein (12 mg/mL) and 75 nanoliter well solution was equilibrated against well solution containing 25% PEG 3350, 0.2 M ammonium sulfate, 17% glycerol. The crystal was mounted directly from the drop and flash-cooled in liquid nitrogen.
NMR Spectroscopy:
Data Collection: Resolution: 2.0Å; X-ray source: Synchrotron SLS-X10SA
Data Processing: