GPX5: Human glutathione peroxidase 5

PDB:2I3Y

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:
Entry Clone Source: Codon optimised, protein sequence derivied from NP_001500
SGC Clone Accession:
Tag: Tag sequence: mhhhhhhssgvdlgtenlyfqs*(m), TEV-cleavable (*), N-terminal his6 tag.
Host: Rosetta-R3

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfqsmKMDCHKDEKGTIYDYEAIALNKNEYVSFKQYVGKHILFVNVATYCGLTAQYPELNALQEELKPY
GLVVLGFPCNQFGKQEPGDNKEILPGLKYVRPGGGFVPSFQLFEKGDVNGEKEQKVFSFLKHSCPHPSEILGTFKSISWDPVKVHDI
RWNFEKFLVGPDGIPVMRWSHRATVSSVKTDILAYLKQFKT

Vector:pNIC-Bsa4

Growth

Medium:
Antibiotics:
Procedure: Medium: TB + 50 µg/ml Kanamycin + 34 µg/ml chloramp .
2 x 1 liter TB in 2.5-L baffled flasks were inoculated with 2 x 10 ml overnight culture and grown at 37°C. The protein expression was induced with 1 mM IPTG at OD600 = 5.5 at 18°C over night. The cells were collected by centrifugation and frozen at -80°C.

Purification

Procedure
Column 1 : Ni-affinity, HisTrap, 1 ml (GE/Amersham Biosciences)
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 A280nm was automatically collected.

Column 2: Hiload 16/60 Superdex 200 prep grade 120 ml (GE/Amersham Biosciences)
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

Concentration : The protein was concentrated in Amicon (5 K) to 8 mg/ml. The protein concentration was determined spectrophotometrically using the predicted molar extinction coefficient 36900(M-1 cm-1).

Extraction

Procedure
Frozen cell pellets from 2 liter were thawed at 37°C and resuspended in a total volume of 100 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 40 000 x g. The supernatant was further clarified by filtration (0.20 m m).
Concentration:
Ligand
MassSpec: The mass determined for GPX 5Ap004 was 24550.2 Da, in agreement with the predicted mass of 24550 for the his-tagged protein.
Crystallization: Crystals were grown by vapor diffusion at 20°C. A sitting drop consisting of 200 nl protein and 100 nl well solution was equilibrated against well solution containing 20% PEG 3350, 200 mM sodium formate, 5 % ethylene glycol. The crystal was transferred to a cryoprotectant composed of 19 % glycerol before flash-cooling in liquid nitrogen.
NMR Spectroscopy:
Data Collection: Resolution: 1.8 Å; X-ray source: Synchrotron SLS -X10, single wavelength.
Data Processing: