GPX1

PDB:2F8A

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:BC000742
Entry Clone Source: Invitrogen clone IOH4675
SGC Clone Accession:
Tag:mhhhhhhssgvdlgtenlyfqs*(m), TEV-cleavable (*), N-terminal his6 tag.
Host: Rosetta-R3

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfqsmQSVYAFSARPLAGGEPVSLGSLRGKVLLIENVASLGGTTVRDYTQMNELQRRLGPRGLVVLGFP
CNQFGHQENAKNEEILNSLKYVRPGGGFEPNFMLFEKCEVNGAGAHPLFAFLREALPAPSDDATALMTDPKLITWSPVCRNDVAWNF
EKFLVGPDGVPLRRYSRRFQTIDIEPDIEALLSQ

Vector:pNIC28-BSA4.

Growth

Medium:
Antibiotics:
Procedure: Medium: TB + 50 Âµg/ml Kanamycin + 34 ug/ml chloramp . 2 x 1 liter TB in 2.5-L baffled flasks were inoculated with 10 ml overnight culture and grown grown at 37Â°C. The protein expression was induced with 1 mM IPTG at OD600 = 4 at 18Â°C overnight . The cells were collected by centrifugation and frozen at -80Â°C.

Purification

Procedure
Column 1 : Ni-affinity, HisTrap, 1 ml (GE/Amersham Biosciences)

Buffers: Lysis buffer: 50 mM potassium phosphate buffer, pH 7.5, 500 mM NaCl, 10 mM imidazole, 0.5 mM TCEP. Wash buffer: 50 mM potassium phosphate buffer, pH 7.5, 500 mM NaCl, 50 mM imidazole, 0.5 mM TCEP. Elution buffer: 50 mM potassium phosphate buffer, pH 7.5, 500 mM NaCl, 250 mM imidazole, 0.5 mM TCEP.

Procedure: 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.

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

Buffers : 10 mM HEPES, pH 7.5, 500 mM NaCl, 5% glycerol, 0.5 mM TCEP

Procedure: The eluted fractions from the Ni-affinity Histrap column were loaded on the gel filtration column at 1.0 ml/min. Eluted proteins were collected in 2 ml fractions

Concentration: The protein was concentrated in Amicon (5 K) to 25 mg/ml and the protein concentration determined spectrophotometrically using the predicted molar extinction coefficient 17780 (M -1 cm -1).

Extraction

Procedure
Lysis buffer: 50 mM potassium phosphate buffer, pH 7.5, 500 mM NaCl, 10 mM imidazole, 0.5 mM TCEP, CompleteÂ® protease inhibitors (1 tablet/50 ml). Frozen cell pellets were thawed on ice overnight and resuspended in a total volume of 50 ml lysis buffer. The cells were disrupted by high pressure (20 kpsi) followed by sonication. Nucleic acids and cell debris were removed by adding 0.15% PEI , followed by centrifugation for 30 minutes at 40.000xg. The supernatant was further clarified by filtration (0.45 m m).
Concentration:
MassSpec:
Crystallization: Column 1 : Ni-affinity, HisTrap, 1 ml (GE/Amersham Biosciences)

Buffers: Lysis buffer: 50 mM potassium phosphate buffer, pH 7.5, 500 mM NaCl, 10 mM imidazole, 0.5 mM TCEP. Wash buffer: 50 mM potassium phosphate buffer, pH 7.5, 500 mM NaCl, 50 mM imidazole, 0.5 mM TCEP. Elution buffer: 50 mM potassium phosphate buffer, pH 7.5, 500 mM NaCl, 250 mM imidazole, 0.5 mM TCEP.

Procedure: 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.

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NMR Spectroscopy:
Data Collection:
Data Processing: