

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:mhhhhhssgvdlgtenlyfqs*(m), TEV-cleavable (*), N-terminal his6 tag.

Host:Rosetta-R3

Construct

Prelude:

Sequence:

mhhhhhssgvdlgtenlyfqsQSVYAFSARPLAGGEPVSLGSLRGKVLLENVASLGGTTVRDYTMNELQRRLGPRGLVVLGFP
CNQFGHQENAKNEEILNSLKYVRPGGGFEPNFMLFEKCEVNGAGAHPLFAFLREALPAPSDATALMTDPKLITWSPVCRNDVAWNF
EKFLVGPDPVPLRRYSRRFQTDIEPDIEALLSQ

Vector:pNIC28-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 10 ml overnight culture and grown grown at 37°C. The protein expression was induced with 1 mM IPTG at OD600 = 4 at 18°C overnight . The cells were collected by centrifugation and frozen at -80°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, Hiloal 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, Complete® 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: