GPX3

PDB:2R37

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:gi|6006001
Entry Clone Source:MGC collection, active-site mutant: Se-Cys (U) was replaced by G
SGC Clone Accession:GPX3A-c068
Tag:C-terminal TEV-cleavable (at *) his-tag with the following sequence AENLYFQ*SHHHHHHDYKDDDDK
Host:BL21(DE3)-R3-pRARE2

Construct

Prelude:
Sequence:

MQEKSKMDCHGGISGTIYEYGALTIDGEEYIPFKQYAGKYVLFVNVASYGGLTGQYIELNALQEELAPFGLVILGFPCNQFGKQEPG
ENSEILPTLKYVRPGGGFVPNFQLFEKGDVNGEKEQKFYTFLKNSCPPTSELLGTSDRLFWEPMKVHDIRWNFEKFLVGPDGIPIMR
WHHRTTVSNVKMDILSYMRRQAALGVAENLYFQ*SHHHHHHDYKDDDDK

Vector:pNIC-CTHF

Growth

Medium:TB
Antibiotics:
Procedure:10ml of overnight culture was added into 1L TB with 50 µg/ml of Kanamycin & 34 µg/ml of Chloramphenicol (total 3 L). The cells were cultured at 37°C until the OD reached 1.511 and then decreased the temperature to 18°C. IPTG was added at 0.5mM (final concentration) and kept the culture at 18°C for overnight.

Purification

Procedure
Column 1 : Ni-Sepharose
Column 2 : Ni-Sepharose

The column was packed by 4 ml of Ni-NTA slurry and equilibrated with 15 ml of binding buffer. The supernatant was loaded onto the column and the flow through was collected. The column was washed with 50 ml of washing buffer I and then 5 ml of washing buffer II. The protein was eluted with 5 ml of elution buffer I and II respectively and 8ml of elution buffer III.

Enzymatic treatment: 100 m l of TEV protease (6mg/ml) were added into the sample. The sample was incubated at 4°C overnight. His-tag was cleaved by TEV protease. The sample was loaded onto the column (packed from 0.4 ml 0f Ni-Sepharose slurry). The flow through was collected and the column was then washed with 3 mls of the buffers with 5, 10 & 20 mM Imidazole (also collected).

Extraction

Procedure
The cells were harvested by centrifugation at 4,000 g for 10 min. The pellet from 1 L culture was resuspended in 25 ml of extraction buffer. The sample was homogenized by using the EmulsiFlex-05 homogenizer (Glen Creston) and then centrifuged at 37505 g. The supernatant was kept for further purification.
Concentration:36.34 mg/ml.
Ligand
MassSpec:23560.1 (23527 expected)
Crystallization: Crystals were grown by vapor diffusion at 4°C in 150nl sitting drops. The drops were prepared by mixing 100nl of protein solution and 50nl of precipitant consisting of 0.1M PCB pH 8.0, 60% MPD. Crystals were flash-cooled in liquid nitrogen.
NMR Spectroscopy:
Data Collection:1.85 Å; X-ray source: Synchrotron SLS-X10SA.
Data Processing: