ZGPAT

PDB:4II1

Revision

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Entry Clone Accession:Q8N5A5
Entry Clone Source:MGC : AT56-C11
SGC Clone Accession:ZGPAT _27 (JMC041:B06): E120-P268
Tag:N-terminal tag: MHHHHHHSSGRENLYFQG
Host:E. coli BL21(DE3)-V2R-pRARE2

Construct

Prelude:
Sequence:

MHHHHHHSSGRENLYFQG EEEGEDEEELSGTKVSAPYYSSWGTLEYHNAMVVGTEEAEDGSAGVRVLYLYPTHKSLKPCPFFLEGK
CRFKENCRFSHGQVVSLDELRPFQDPDLSSLQAGSACLAKHQDGLWHAARITDVDNGYYTVKFDSLLLREAVVEGDGILPP

Vector:pET28-MHL

Growth

Medium:
Antibiotics:
Procedure:A fresh transformation was used to inoculate 50 mL LB media containing 50 µg/mL kanamycin and 30 µg/mL chloramphenicol The culture was grown overnight at 37°C with shaking. The next day this starter culture was used to inoculate 2L of TB growth medium. The culture was grown in LEX at 37°C to OD600 of 1. IPTG-based induction was carried out according to the manufacturer's protocol. The temperature was reduced to 14°C and the culture was incubated for a further 18 hours before harvesting the cells.

Purification

Procedure
IMAC: Unclarified lysate was mixed with 2 mL of Ni-NTA superflow Resin (Qiagen) per 40 mL lysate. The mixture was incubated with mixing for at least 45 minutes at 4oC. The mixture was then loaded onto an empty column (BioRad) and washed with 50 mL wash buffer. Samples were eluted from the resin by exposure to 2-3 column volumes (approx. 10-15 mL) of elution buffer. Concentration of eluted protein was estimated by OD280.

Gel filtration chromatography: An XK 26x65 column (GE Healthcare) packed with HighLoad Superdex 75 resin (GE Healthcare) was pre-equilibrated with gel filtration buffer for 1.5 column volumes using an AKTA explorer (GE Healthcare) at a flow rate of 1.0 mL/min. The dialyzed sample from the IMAC step (approx. 15 mL) was loaded onto the column at 1.5 mL/min, and 2mL fractions were collected into 96-well plates (VWR 40002-012) using peak fractionation protocols. Fractions observed by a UV absorption chromatogram to contain the protein were pooled.

Extraction

Procedure
Cells were harvested by centrifugation and pellets were stored in -80ºC. Prior to purification, the cell pellet was resuspended in lysis buffer. Cells were disrupted by sonication (10 minutes) and samples were centrifuged for 60 min at 70000 g.
Concentration: Purified proteins were concentrated using 15 mL concentrators with a 3,000 molecular weight cut-off (Amicon Ultra-15, UFC900524, Millipore) at 3750 rpm, typically resulting in a final concentration around 10mg/mL.
Ligand
MassSpec:
Crystallization: Recombinant human Zinc finger domain of ZGPAT was crystallized using the sitting dropvapour diffusion method at 18 °C. The crystals were obtained in a buffer containing. Crystals were soaked in a cryoprotectant consisting of 100% reservoir solution and 15% glycerol.
NMR Spectroscopy:
Data Collection:
Data Processing: