C1QBP

PDB:3RPX

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:BC000435
Entry Clone Source:MGC:AU1-D11
SGC Clone Accession:HPC09Q-F01
Tag:C-terminal His6-tag
Host:BL21(DE3)-V2R-pRARE2

Construct

Prelude: Construct ID C1QBP:P3-Q282:CH
The construct was designed to encode residues P3-Q282. However, DNA sequence encoding residues from a.a. 18 to 88 was skipped in the PCR products, probably due to strange DNA secondary structure and the resulting plasmid encodes a truncated protein of E89-Q282 and the reading frame was not right.
The measured mass of the purified protein was 22119.6, the closest construct will be I96-Q282 (m.w. expected 22102.23).
Sequence:

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IQKHKTLPKMSGGWELELNGTEAKLVRKVAGEKITVTFNINNSIPPTFDGEEEPSQGQKVEEQEPELTSTPNFVVEVIKNDDGKKAL
VLDCHYPEDEVGQEDEAESDIFSIREVSFQSTGESEWKDTNYTLNTDSLDWALYDHLMDFLADRGVDNTFADELVELSTALEHQEYI
TFLEDLKSFVKSQahhhhhhThe exact N-terminus is not clear
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Vector:pNIC-CH

Growth

Medium: Terrific Broth medium in the presence of 50 mg/mL kanamycin and 25 mg/mL chloramphenicol
Antibiotics:
Procedure:LEX Bubbling. The target protein was expressed in *E. coli* by inoculating 100 mL of overnight culture grown in Luria-Bertani medium into a 2 L of growth medium at 37 °C. When OD_{600} reached ~3.0, the temperature of the medium was lowered to 15 °C and the culture was induced with 1 mM IPTG. The cells were allowed to grow overnight before harvested and flash frozen in liquid nitrogen and stored at -80 °C.

Purification

Procedure

The lysate was centrifuged at 16,000 rpm for 60 minutes and the supernatant were mixed with 6 mL 50% flurry of Ni-NTA beads and incubated at 4 degrees Celsius on rotary shaker for 60 minutes. The mixture was then centrifuged at 2300 rpm for 5 min and the supernatant discarded. The beads were then washed with 100 mL binding buffer containing 5 mM Imidazole followed by 50 mL washing buffer. Bound proteins were eluted using 15 mL elution buffer. The flow-through was collected and further purifed by a Superdex-75 26/60 gel filtraton column pre-equilibrated with gel filtration buffer. Fractions containing the target protein were pooled and concentrated using Amicon Ultra-15 centrifugal filter (mwco 10 kDa). The purity of the preparation is tested by SDS-PAGE to be greater than 90%.

Extraction

Procedure
Frozen cells from 4L TB culture were thawed and resuspended in 500 mL extraction buffer with freshly added 0.5% CHAPS, and supplemented with 1.8 mL protease inhibitor cocktail (SIGMA Catalog # P8849), and 10 uL benzonase (Sigma Catalog # E1014, 250U/uL), 1mM PMSF/Benzamidine, and lysed using sonication for 8 min at 120 W, 10 sec on/10 sec off duty cycle.
Concentration:24 mg/mL
Ligand
MassSpec: Native protein measured 22119.6
Crystallization: Crystal was initially obtained from SGC-I screen condition H7 (also available from SGC:A8,D6).
Crystal used for structure refinement was grown in 25% PEG 1500, 0.2 M NaCl, 0.1 M HEPES buffer pH 7.5, 5% Glycol sitting drop setup, using 0.3 uL protein + 0.3 uL well solution against 100 uL reservoir buffer at room temperature.

Crystals grow to a mountable size within a few days.
Cryo used paratone-N
NMR Spectroscopy:
Data Collection:
Data Processing: