

ZCWPW2

PDB:4Z0O

Revision

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Entry Clone Accession:NP_001035522.1.

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SGC Clone Accession:ZCWPW2_9 (JMC01B:H12): V21-F78 with F78D mutation

Tag:N-terminal tag: MHHHHHHSSGRENLYFQG

Host:Escherichiacoli BL21 (DE3)

Construct

Prelude:

Sequence:

MHHHHHHSSGRENLYFQGVENMYVNKVWVQCENENCLKWRLSSEDSAKVDHDEPWYCFMNTDSRYNNCSISEEDD

Vector:pET28-MHL

Growth

Medium:

Antibiotics:

Procedure:Lex system, 37 °C 3-4h, 15 °C 18-24h

Purification

Procedure

IMAC: Unclarified lysate was mixed with 3-4 mL of Ni-NTA superflow Resin (Qiagen) per 200 mL lysate. The mixture was incubated with mixing for at least 30 minutes at 4°C. The mixture was then loaded onto an empty comLum (BioRad) and washed with 40 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 16x60 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 concentrate sample (approx. 3 mL, concentrated by using 15 mL concentrators with a 3,000 molecular weight cut-off (Amicon Ultra-15, UFC900524, Millipore) at 3750 rpm) from the IMAC step was loaded onto the column at 1 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

Frozen cell pellet contained in bags (Beckman 369256) obtained from 2L of culture were thawed by soaking in warm water. Each cell pellet was resuspended in 200 mL lysis buffer and homogenized using an Ultra-Turrax T8 homogenizer (IKA Works) at maximal setting for 30-60 seconds per pellet. Cell lysis was accomplished by sonication (Virtis408912, Virsonic) on ice: the sonication protocol was 10 seconds pulse at 80% of maximal frequency (8.0), 10 seconds rest, for 10 minutes total sonication time per pellet.

Concentration: Purified proteins were concentrated using 15 mL concentrators with a 5,000 molecular weight cut-off (Amicon Ultra-15, UFC900524, Millipore) at 3750 rpm, typically resulting in a final concentration around 20 mg/mL.

Ligand

MassSpec:

Crystallization: Recombinant human ZCWPW2 CW zinc finger with F78D mutation was mixed with H3K4me3 peptide at molar ratio of 1:3 and co-crystallized using the sitting drop vapour diffusion method at 18 °C. The crystals were obtained in a buffer containing 2 M NH₄SO₄, 2% PEG 400, 0.1 M Na Hepes, pH 7.5. Crystals were soaked in a cryoprotectant consisting of 100% reservoir solution and 15% glycerol.

NMR Spectroscopy:

Data Collection:

Data Processing: