

MPDZ

PDB:2FCF

Revision

Revision Type:created

Revised by:created

Revision Date:created

Entry Clone Accession:MPDZA-s001

Entry Clone Source:Origene

SGC Clone Accession:

Tag:N-terminal hexahistidine tag before TEV cleavage site and C-terminal PDZ recognition motif: **STRL**

Host:BL-21(DE3)R3 phage resistant

Construct

Prelude:

Sequence:

mhahhhhhssggvdlgtenlyfqsmQPRRVELWREPSKSLGISIVGGRGMGSRLSNGEVMRGIFIKHVLEDSPAGKNGTLKPGDRIVEV
DGMDLRDASHEQAVEAIRKAGNPVVFMVQSIIstr1

Vector:pNIC28-BSA4.

Growth

Medium:

Antibiotics:

Procedure:Transformed 50 μ l competent BL-21 (DE3) phage resistant cells with 10 μ l of the plasmid DNA and plated out onto LB plate plus 50 μ g/mL kanamycin. The next day colonies were picked out into fresh deep well blocks containing 1 mL TB + 50 μ g/mL kanamycin. These were grown overnight and glycerol stocks prepared by adding 333 μ l of 60 % glycerol to 1 mL of cell suspension, mixing and then storing in a -80°C freezer.

Purification

Procedure

Column 1 : Ni-affinity, HisTrap, 1 mL (GE/Amersham)

Buffers: Affinity binding buffer: 10mM Imidazole, 300mM NaCl, 50mM pH8.0 NaH2PO4, 0.5mM TCEP. Affinity wash buffer: 50mM Imidazole, 300mM NaCl, 50mM pH8.0 NaH2PO4, 0.5mM TCEP. Affinity Elution Buffer: 250mM Imidazole, 300mM NaCl, 50mM pH8.0 NaH2PO4, 0.5mM TCEP

Procedure: The cell extract was loaded on the column at 0.8 mL/min on an AKTA-express system (GE/Amersham). The column was then washed with 10 column volumes of Affinity Binding buffer, 10 column volumes of Affinity wash buffer, and then eluted with Affinity elution buffer at 0.8 mL/min. The eluted peak of A280 was automatically collected.

Column 2: Gel filtration, Hiload 16/60, S75 16/60 - 120 mL

Buffers : Gel Filtration: 10mM pH7.4 Hepes, 500mM NaCl, 5% glycerol, 0.5mM TCEP

Procedure: The eluted fractions from the Ni-affinity Histrap columns were loaded on the gel filtration column in GF buffer at 1.0 mL/min. Eluted proteins were collected in 1 mL fractions.

Concentration: Using a Centricon 10 K cutoff concentrator the MPDZA-p065 pooled fractions was concentrated to 57.6 mg/mL. Concentration was determined from the absorbance at 280 nm.

Extraction

Procedure

Lysis buffer: 10mM Imidazole, 300mM NaCl, 50mM pH8.0 NaH₂PO₄, 0.5mM TCEP, 1x complete PI EDTA free tablet/50mLs. The pellet (19.52 gms) was resuspended with 3x volume of lysis buffer (approximately 50 mLs final) by intermittently placing the pellet in a 37°C water bath and vortexing. Once resuspended the cells were (1) broken by one passage through the Constant Systems cell breaker; (2) sonicating; (3) DNA precipitation with the addition of PEI to a final concentration of 0.15 % for 30 mins on ice followed by a 17,000 rpm at 4°C to remove precipitation; (4) the supernatant was filtered through a GF/0.2 μM serum acrodiscs.

Concentration:

MassSpec:

Crystallization: Column 1 : Ni-affinity, HisTrap, 1 mL (GE/Amersham)

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NMR Spectroscopy:

Data Collection:

Data Processing: