

# 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:**

mhhhhhhssgvdlgtenlyfqsmQPRRVELWREPSKSLGISIVGGRMGSRLSNGEVMRGIFIKHVLEDSPAGKNGTLKPGDRIVEV  
DGMDLRDASHEQAVEAIRKAGNPVVMVQSIIstr1

**Vector:**pNIC28-BSA4.

## Growth

**Medium:**

**Antibiotics:**

**Procedure:**Transformed 50  $\mu$ l competent BL-21 (DE3) phage resistant cells with 10  $\mu$ l of the plasmid DNA and plated out onto LB plate plus 50  $\mu$ g/mL kanamycin. The next day colonies were picked out into fresh deep well blocks containing 1 mL TB + 50  $\mu$ g/mL kanamycin. These were grown overnight and glycerol stocks prepared by adding 333  $\mu$ 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 NaH<sub>2</sub>PO<sub>4</sub>, 0.5mM TCEP. Affinity wash buffer: 50mM Imidazole, 300mM NaCl, 50mM pH8.0 NaH<sub>2</sub>PO<sub>4</sub>, 0.5mM TCEP. Affinity Elution Buffer: 250mM Imidazole, 300mM NaCl, 50mM pH8.0 NaH<sub>2</sub>PO<sub>4</sub>, 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 NaH<sub>2</sub>PO<sub>4</sub>, 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)

Buffers: Affinity binding buffer: 10mM Imidazole, 300mM NaCl, 50mM pH8.0 NaH<sub>2</sub>PO<sub>4</sub>, 0.5mM TCEP. Affinity wash buffer: 50mM Imidazole, 300mM NaCl, 50mM pH8.0 NaH<sub>2</sub>PO<sub>4</sub>, 0.5mM TCEP. Affinity Elution Buffer: 250mM Imidazole, 300mM NaCl, 50mM pH8.0 NaH<sub>2</sub>PO<sub>4</sub>, 0.5mM TCEP

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### **NMR Spectroscopy:**

### **Data Collection:**

### **Data Processing:**