MPDZA: Human multiple PDZ domain protein (12th domain)

PDB:2IWO

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:MPDZA-s001
Entry Clone Source: Origene
SGC Clone Accession:
Tag:
Host:E.coli BL-21(DE3)R3 phage resistant

Construct

Prelude:mhhhhhhssgvdlgtenlyfqsm - hexahistadine tag followed by a TEV cleavage site plus additional residues due to construct design. C-terminal PDZ binding motif (ETSV)
Sequence:

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mhhhhhhssgvdlgtenlyfqsmGLRTVEMKKGPTDSLGISIAGGVGSPLGDVPIFIAMMHPTGVAAQTQKLRVGDRIVTICGTSTE
GMTHTQAVNLLKNASGSIEMQVVAGGDVSETSV
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Vector:pNIC28-Bsa4

Growth

Medium:
Antibiotics:
Procedure: Using a glycerol stock of the transformed cells, a starter culture (20 ml of LB plus 50µg/ml kanamycin) was grown overnight. This was used to inoculate 2 litres of Terrific Broth plus 50µg/ml kanamycin. The cells were further grown at 37°C (200 rpm) until the culture reached an OD600 of ~1.0. At this stage the temperature of the incubator was dropped to 25°C for 1 hour before induction of protein synthesis by the addition of 1 mM IPTG. Total induction time was 16 hour after which the cells were pelleted by centrifugation at 4000 rpm for 10 minutes at 4°C. The pellets were then resuspended in 25 mls of Resuspension Buffer and stored in a -80°C freezer unitil further use. Resuspension Buffer: 50mM Hepes pH 8.0, 150mM NaCl, 5% Glycerol, 5mM Imidazole pH 8.0, 0.5mM TCEP.

Purification

Procedure

Column 1 : Ni-affinity, HisTrap, 1 ml (GE/Amersham)
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, the 10 column volumes of Affinity Wash Buffer II and the MPDZ PDZ domain 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
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 : The eluted fractions that contained the PDZ domain as judged by SDS - PAGE were pooled and concentrated to 32 mg/ml using a 10 kD cutoff spin concentrator. The concentrated protein was distributed into 7 x 50 µl aliquots before freezing in a -80°C freezer.

Extraction

Procedure
One EDTA-free protease table (Roche) was dissolved in 10 mls of Resuspension Buffer and added to each of the frozen cell suspensions (estimated total volume 45 mls). Aftr thawing the cells were broken open by passing 5 times through an Emulsiflex C5 high pressure homogeniser (Avestin). Insoluble material was removed by centrifugation at 16,000 rpm for 40 minutes at 4°C.
Concentration:
Ligand
MassSpec: Before His-Tag removal: Expected MWt. = 12535.1; Measured MWt. = 12534.9.
Crystallization: Two crystal forms grew with different space groups that were acceptable for structure determination. They were grown under the following conditions:

P21212 crystals (PDB: 2IWO) grew from a 2:1 ratio mix of MPDZA 12th PDZ domain-to-reservoir (2 M $(NH_4)_2SO_4$).

C222 1 crystals (PDB: 2IWP) grew from a 1:1 ratio mix of MPDZA 12th PDZ domain-to-reservoir (50 % PEG 300 ; 0.2 M Li_2SO_4; 0.1 M acetate pH 4.5).
NMR Spectroscopy:
Data Collection: Resolution: 1.70 Å (2IWO) and 2.15Å (2IWP);X-ray source: Synchrontron SLS-X10.
Data Processing: