Entry Clone Source: Origene

Entry Clone Accession: NM_003829 Variant

SGC Construct ID: MPDZA-c145

GenBank GI number: gi|4505231

Vector: pNIC28-Bsa4. Details [PDF]; Sequence [FASTA] or [GenBank]

Tags and additions:
mhhhhhhssgvdlgtenlyfqsmGLR
TVEMKKGPTDSLGISIAGGVGSPLGDVPI
FIAMMHPTGVAAQTQKLRVGDRIVTICGT
STEGMTHTQAVNLLKNASGSIEMQVVAGG
DVS**ETSV**

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

Host : *E.coli* BL-21(DE3)R3 phage resistant

Growth medium, induction protocol: 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 OD_{600} 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.

Extraction buffer, extraction method: One EDTA-free protease inhibitor tablet (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.

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

Buffers: Affinity wash buffer: 50 mM HEPES pH 7.5, 10 mM Imidazole, 100 mM NaCl, 0.5 mM TCEP, 2 mM $MgCl_2$, 5 % Glycerol; **Affinity Wash Buffer II:** 50 mM HEPES pH 7.5, 100 mM NaCl, 5 % Glycerol, 2 mM $MgCl_2$, 30 mM Imidazole pH 8.0, 0.5 mM TCEP; **Affinity Elution Buffer:** 50 mM potassium phosphate pH 7.4, 250 mM Imidazole, 500 mM NaCl, 0.5 mM TCEP, 2 mM $MgCl_2$, 5 % Glycerol.

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 A_{280} was automatically collected.

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

Buffers : Gel Filtration: 50 mM HEPES pH 7.5, 100 mM NaCl, 2 mM $MgCl_2$, 0.5 mM 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 : 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.

Mass spec characterization : Before His-Tag removal: Expected MWt. = 12535.1; Measured MWt. = 12534.9.

Crystallisation: Two crystal forms grew with different space groups that were acceptable for structure determination. They were grown under the following conditions:
$P2_12_12$ crystals (PDB: 2IWO) grew from a 1:2 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).

Data Collection: Resolution: 1.70 Å (2IWO) and 2.15Å (2IWP);**X-ray source:** Synchrotron SLS-X10.