DLG3: First PDZ domain of the human discs, large homolog 3 protein.

PDB:2I1N

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:
Entry Clone Source: Origene
SGC Clone Accession:
Tag:N-terminal hexahistidine tag: mhhhhhhssgvdlgtenlyfq*sm
Host:BL-21(DE3)R3 phage resistant strain

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfq*smFKYEEIVLERGNSGLGFSIAGGIDNPHVPDDPGIFITKIIPGGAAAMDGRLGVNDCVLRVNEV
DVSEVVHSRAVEALKEAGPVVRLVVRRRQPPPEETSV

Vector:pNIC28-Bsa4

Growth

Medium:
Antibiotics:
Procedure: Freshly transformed E. coli cells was used to inoculate 40 ml of LB containing 50 µg/ml kanamycin for overnight growth. The following day, 10 mls of this starter was used to inoculate 1 litre of TB plus 50 µg/ml kanamycin. When OD600 reached ~1.6 the temperature was shifted down from 37°C to 25°C for 1 hour before induction with the addition of 1 mM IPTG. Protein expression was allowed to carry on for a futher 4 hours before harvest. The cells were harvested by centrifugation, resuspended in lysis buffer before storing in a -80°C freezer.

Purification

Procedure

Extraction

Procedure
To the cell pellet (approx. 80 mls) was added 20 mls of Lysis/Binding buffer and PMSF was added to a final concentration of 1.0 mM. Cell breakage: 4 passes through the Emulsiflex C5 high pressure homogeniser. Total vol: 100 mls (estimate).PEI was added to a final concentration of 0.05 % to precipitate the DNA .Centrifuge for 40 mins at 18000 rpm and 4°C to remove cell debris.Discard pellet.
Concentration:
Ligand
MassSpec: Expected 10918.3; recorded 12602.49.
Crystallization: Crystals grew from a 2:1 mixture of DLG3-to-reservoir (1.0 M LiSO4, 0.5 M TMAO).
NMR Spectroscopy:
Data Collection: Resolution: 1.1 Å; X-ray source: Synchrotron SLS -X10, single wavelength.
Data Processing: