DLG2

PDB:2HE2

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:DLG2A_s001
Entry Clone Source: Origene
SGC Clone Accession:
Tag:N-terminal hexahistidine tag before TEV cleavage site C-terminal PDZ recognition motif (ETSV).
Host:

Construct

Prelude:sm residues are a result of the construct design
Sequence:

```
sm EPRKVVLHKGSTGLGFNIVGGEDGEGI FVSFILAGGPADLSGELQRGDQILSVNGI DLRGASHEQAAAALKGAGQTVTIIAQ
YQP EDYARFEAKIHETSV
```

Vector:pNIC28-Bsa4

Growth

Medium:
Antibiotics:
Procedure: Transformed 90 µl competent BL-21 (DE3) phage resistant cells with 2 µl of the plasmid DNA and plated out onto LB plate plus 50 µg/ml kanamycin and 34µg/ml chloramphenicol. The next day colonies were picked out into 50 ml LB + 50 µg/ml kanamycin. 15 ml of the culture was used to innoculate 1 litre of TB + 50 µg/ml kanamycin and 34µg/ml chloramphenicol. When the OD600 reached 1.4 the incubator temperature was dropped to 25°C. Protein production was induced with the addition of 1mM IPTG one hour after the temperature shift. After 4 hours cells were harvested by centrifugation at 4000 rpm for 15 minutes resuspended in Lysis Buffer before storage in the -80°C freezer.

Purification

Procedure
Column 1 : Ni^{2+}-NTA column purification
1 ml of 50 % Ni2+-NTA slurry was added to a clean empty 10 mm diameter gravity column. The resin was equilibrated with 10 ml Lysis/Binding buffer (see above). The supernatant was allowed to drip through the resin twice. The resin was then washed with 50 mls of Lysis Buffer and 30

mls of Wash Buffer. Finally the protein was eluted with 15 mls of Elute buffer with 2 ml fractions collected into eppendorf tubes.

Column 2: Gel filtration, Hiload 16/60, S75 16/60 - 120 ml
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. The fractions containing protein were identified on a Coomassie blue stained SDS - PAGE gel.

Enzymatic treatment : Added 100 µl of the home produced TEV protease to fractions containing protein and left at 4°C overnight. The following steps were carried out to remove the cleaved products and TEV protease. Place 200 µl of 50 % Ni-NTA agarose in a 1.5 ml eppendorf tubes, add 1 ml of 50 mM HEPES pH 7.5, 100 mM NaCl, 0.5 mM TCEP, 2 mM MgCl2 mix, spin down and remove buffer. Repeat this resin wash step once. Add the TEV treated protein sample to the resin and mix for 30 min. Finally spin down resin and collect the supernatant which contains the cleaved DLG2 PDZ domain. The protein was then concentrated to 4 mg/ml before aliquoted into 50 µl volumes and storing in a -80°C freezer.

Extraction

Procedure
Once the cells were thawed they were broken by several passages through the Avestin C5 high pressure homogeniser. The final broken cells suspension came to an approx. volume of 50 mls. To this 0.15 % of PEI was added to precipitate the DNA before centrifugation at 21,500 rpm for 45 mins at 4°C to remove the precipitated insoluble material.
Concentration:
Ligand
MassSpec: Expected MWt. after his-tag removal: 10665.8; Measured MWt.:10665.
Crystallization: Crystals grew from a 2:1 ratio mix of DLG2-to-reservoir (25.5 % PEG 3350 , 0.17 M (NH4)2SO4, 15 % glycerol)
NMR Spectroscopy:
Data Collection: Resolution: 1.50Å; X-ray source: Synchrontron SLS-X10.
Data Processing: