

DLG3-PDZ domain

PDB:2FE5

Revision

Revision Type:created

Revised by:created

Revision Date:created

Entry Clone Accession:DLG3A-s001

Entry Clone Source:Origene

SGC Clone Accession:

Tag:N-terminal hexahistidine tag

Host:BL-21(DE3)R3 phage resistant strain

Construct

Prelude:

Sequence:

mhahhhhhsgvdlgtenlyfq*smTIMEVNLLKGPKGLGFSIAGGGIGNQHIPGDNSIYITKIIEGGAAQKDGRQLIGDRLLAVNNTN
LQDVRHEEAVASLKNTSDMVKVAKPGS

Vector:pNIC28-Bsa4

Growth

Medium:

Antibiotics:

Procedure:Freshly transformed E. coli cells was used to inoculate 40 ml of LB containing 50 μ g/ml kanamycin for overnight growth. The following day, 10 mls of this starter was used to inoculate 1 litre of TB plus 50 μ g/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.

Lysis/Binding Buffer: 50 mM Hepes pH 8.0, 500 mM NaCl, 5 % glycerol, 5 mM imidazole pH 8.0, 0.5 mM TCEP.

Purification

Procedure

Column 1 : Wash Buffer: 50 mM Tris pH 8.0, 500 mM NaCl, 5 % Glycerol, 25 mM Imidazole pH 8.0, 0.5 mM TCEP; Elute Buffer: 50 mM Hepes pH 8.0, 500 mM NaCl, 5 % Glycerol, 250 mM Imidazole pH 8.0, 0.5 mM TCEP; GF Buffer: 50 mM Tris pH 8.5, 500 mM NaCl, 0.5 mM TCEP.

Procedure: The supernatent was filtered through a 1.2 micron and then a 0.2 micron syringe filter.

4 mls of a 50 % Ni2+-NTA slurry was added to a clean 15 mm diameter gravity column. The resin was equilibrated with 5 column volumes (10 ml) Lysis/Binding buffer. The supernatant was allowed to drip through the resin twice. The resin was then washed with 5 column volumes of Lysis/Binding Buffer and 25 column volumes of Wash Buffer. Finally the protein was eluted with 6 column volumes of Elute buffer and the eluate collected in 2ml fractions into eppendorf tubes. Enzymatic treatment : HIS- TAG removal. To improve the purity of the sample the hexahistidine tag was cleaved and the cleavage products plus the contaminants rebound to a Ni2+-resin thus allowing the cleaved DLG3A sample to run through the column.

The DLG3A-p032 fractions were pooled and cleaved overnight with 100 μ l of 2.8 mg/ml of in-house produced TEV protease. 500 μ l of 50 % Ni-sepharose (Amersham) was added and the sample inverted repeatedly on a rotator for 60 minutes at 4°C to remove the His tag with TEV and other contaminants. The protein was concentrated to a final concentration of 143 mg/ml before storing at -80°C.

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:

Crystallization:Column 1 : Wash Buffer: 50 mM Tris pH 8.0, 500 mM NaCl, 5 % Glycerol, 25 mM Imidazole pH 8.0, 0.5 mM TCEP; Elute Buffer: 50 mM Hepes pH 8.0, 500 mM NaCl, 5 % Glycerol, 250 mM Imidazole pH 8.0, 0.5 mM TCEP; GF Buffer: 50 mM Tris pH 8.5, 500 mM NaCl, 0.5 mM TCEP.

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NMR Spectroscopy:

Data Collection:

Data Processing: