DLG2

PDB:2BYG

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
Host:BL-21(DE3_R3 phage resistant)

Construct

Prelude:
Sequence:

MHHHHHHSSGVDLGTENLYFQSMTVVEIKLFKGPKGLGFSIAGGVGNQHIPGDNSIYVTKIIDGGAAQKDGRLQVGDRLLMVNNYSL
EEVTHEEAVAILKNTSEVVYLKVGKPTTIY

Vector:pNIC28-Bsa4

Growth

Medium:
Antibiotics:
Procedure: Transformed 50 µL competent BL21 (DE3) phage resistant cells with 10 µL of the plasmid DNA and plated out onto LB plate plus 50 µg/mL kanamycin. The next day colonies were picked out into fresh deep well blocks containing 1 mL TB + 50 µg/mL kanamycin. These were grown overnight and glycerol stocks prepared by adding 333 µL of 60 % glycerol to 1 mL of cell suspension, mixing and then storing in a -80C freezer.

The glycerol stock was used to innoculate 10 mLs of TB + 50 µg/mL kanamycin which was grown overnight at 37C as a starter culture for a 1 litre growth. The large scale growth was grown at 37C until approximately 30 mins before induction when the temperature was lowered to 25C. Protein production was induced with the addition of 1mM IPTG. The next day cells were harvested by centrifugation at 4000 rpm for 15 minutes. The pellet was then stored in the -80C freezer.

Purification

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

Buffers: Affinity binding buffer: 10 mM Imidazole, 300 mM NaCl, 50 mM pH 8.0 NaH_2PO_4 , 0.5 mM TCEP. Affinity wash buffer: 50mM Imidazole, 300mM NaCl, 50mM pH8.0 NaH2PO4 , 0.5mM TCEP. Affinity Elution Buffer: 250mM Imidazole, 300mM NaCl, 50mM pH8.0 NaH2PO4 , 0.5mM TCEP

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, and then 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

Buffers: Gel Filtration: 10mM pH7.4 Hepes, 500mM NaCl, 5% glycerol, 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: Using a Centricon 10 K cutoff concentrator the DLG2A-p002 pooled fractions was concentrated to 25.8 mg/mL. Concentration was determined from the absorbance at 280 nm.

Extraction

Procedure
Lysis buffer: 10 mM Imidazole, 300mM NaCl, 50mM pH8.0 NaH_2PO_4 0.5 mM TCEP, 1x complete PI EDTA free tablet/50mL. The pellet (19.52 g) was resuspended with 3x volume of lysis buffer (approximately 50 mL final) by intermitently placing the pellet in a 37C water bath and vortexing. Once resuspended the cells were (1) broken by one passage through the Constant Systems cell breaker; (2) sonicating; (3) DNA precipitation with the addition of PEI to a final oncentration of 0.15% for 30 mins on ice followed by a 17,000 rpm at 4C to remove precipitation; (4) the supernatant was filtered through a GF/0.2 μM serum acrodiscs.
Concentration:
Ligand
MassSpec:
Crystallization: Column 1 : Ni-affinity, HisTrap, 1 mL (GE/Amersham)

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NMR Spectroscopy:

Data Collection:

Data Processing: