DUSP16

PDB:2VSW

Revision

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Entry Clone Accession: IMAGE:4400399
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Tag:
Host:BL21 (DE3)R3

Construct

Prelude:
Sequence:

MIGTQIVTERLVALLESGTEKVLLIDSRPFVEYNTSHILEAININCSKLMKRRLQQDKVLITELIQHSAKHKVDIDCSQKVVVYDQS
SQDVASLSSDCFLTVLLGKLEKSFNSVHLLAGGFAEFSRCFPGLCEGKSTLVPTCISQPAHHHHHH

Vector: pNIC-CH

Growth

Medium:
Antibiotics:
Procedure: Growth medium, induction protocol: 1ml from a 10 ml overnight culture containing 50 µg/ml kanamycin was used to inoculate 1 litre of LB containing 50 µg/ml kanamycin. Cultures were grown at 37°C until the OD600 reached ~0.3 then the temperature was adjusted to 18°C. Expression was induced for overnight using 1 mM IPTG at an OD600 of 0.8. The cells were collected by centrifugation and the pellet resuspended in binding buffer and frozen. Binding buffer: 50mM HEPES pH 7.5; 500 mM NaCl; 5 mM imidazole, 5% glycerol; 0.5 mM TCEP.

Purification

Procedure
Column 1: Ion exchange - Nucleic acid removal. DEAE cellulose (DE52, Whatman), 10 g of resin in 2.5 x 20 cm column. The resin was hydrated in 2.5M NaCl, then washed with 20 ml binding buffer prior to loading the sample.Buffers: 50mM HEPES pH 7.5; 500 mM NaCl; 5 mM imidazole, 5% glycerol; 0.5 mM TCEPProcedure: Supernatant was applied by gravity flow, followed by a wash with 100 ml binding buffer. The column flow-through was collected.Column 2: Ni-affinity. Ni-NTA (Qiagen), 5 ml of 50% slurry in 1.5 x 10 cm column, washed with binding buffer.Buffers : Binding buffer: 50 mM HEPES pH 7.5, 500 mM NaCl, 5% Glycerol, 0.5 mM

TCEP; Wash buffer: 50 mM HEPES pH 7.5, 500 mM NaCl, 30 mM Imidazole, 5% glycerol, 0.5 mM TCEP; Elution buffer: 50 mM HEPES pH 7.5, 500 mM NaCl, 50 to 250 mM Imidazole, 5% Glycerol, 0.5 mM TCEP.Procedure: The flowthrough from column 1 was loaded by gravity flow on the Ni-NTA column. The column was then washed with 100 ml wash buffer at gravity flow. The protein was eluted by gravity flow by applying 5-ml portions of elution buffer with increasing concentration of imidazole (50 mM, 100 mM, 150 and 250 mM); fractions were collected until essentially all protein was eluted.Enzymatic treatment : noneColumn 3: Size Exclusion Chromatography. Superdex S200 16/60 HiLoadBuffers: 25 mM HEPES, pH 7.5; 250 mM NaCl, 5 mM DTTProcedure: DUSP16 was concentrated and applied to an S200 16/60 HiLoad gel filtration column equilibrated in 25 mM HEPES, pH 7.5; 250 mM NaCl, 5 mM DTT using either an ÄKTAprime or ÄKTAexpress system.

Extraction

Procedure
Extraction buffer, extraction method: Frozen pellets were thawed and cells lysed using a high pressure cell disrupter. The lysate was centrifuged at 17,000 rpm for 30 minutes and the supernatant collected for purification.
Concentration: Protein concentration: Protein was concentrated to 10 mg/ml using an Amicon 3 kDa cut-off concentrator.
Ligand
MassSpec: Mass spec characterization: LC- ESI -MS TOF gave a measured mass of 17050 for this construct. Predicted mass 17050.
Crystallization: Crystallization: Initial crystals grown in 30% PEG-3350, 0.2M MgCl2, Tris-HCl pH 7.5 were used for seeding. Diffraction quality crystals were grown at 20°C in 150nl drops from a 2:1 ratio of protein to reservoir solution with 15nl of the seed stock. The reservoir solution was 0.20M NaNO3; 0.1M BTProp pH 8.5; 20.0% PEG 3350; 10.0% EtGly.
NMR Spectroscopy:
Data Collection: Data Collection: Crystals were cryo-protected using 25% glycerol. X-ray source: Diffraction data were collected at the SLS beamline X10 at a single wavelength. Resolution: 2.2 Å resolution limit.
Data Processing: