DVL2

PDB:2REY

Revision

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Entry Clone Accession:gi|4758216
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Tag:N-terminal, TEV cleavable hexahistidine tag
Host:BL-21(DE3)-R3-Rosetta

Construct

Prelude:
Sequence:

SMSLNIITVTLNMEKYNFLGISIVGQSNERGDGGIYIGSIMKGGAVAADGRIEPGDMLLQVNDMNFENMSNDDAVRVLRDIVHKPGP
IVLTVAKCWETSV

Vector:pNIC28-Bsa4

Growth

Medium:
Antibiotics:
Procedure: Transformation: The construct DNA was transformed into homemade chemically competent cells of the expression strain by a standard heat shock procedure.Glycerol stock prepataion: A number of colonies from the transformation were used to innoculate 1 ml of LB media containing 50 µg/ml kanamycin and 34 µg/ml chloramphenicol, which was placed in a 37°C shaker overnight. The next day glycerol stocks were prepared from this overnight culture.Expression: 10 ml of a thawed glycerol stock was used to innoculate 40 ml of TB media containing 50 µg/ml kanamycin and 34 µg/ml chloramphenicol, which was placed in a 37°C shaker overnight. The next day this starter culture was used to innoculate 2x 1L of TB media (18 ml starter culture into each) containing 50 µg/ml kanamycin. After 4.5 hours, the temperature was reduced to 22°C. The incubation was continued for 1 hour. At OD~3, the cells were induced by the addition of 0.5 mM IPTG. The expression was continued overnight (~ 18 hours).Cell harvest: Cells were spun at 6238x g for 15 mins at 4°C. The cell pellets were placed in a -80°C freezer.

Purification

Procedure
Column 1: HisTrap 1ml.The clarified cell extract was passed through the column at a flow rate of

0.8 ml/min. The column was then washed with Wash Buffer until a stable UV baseline was achieved. The protein was eluted with 5 ml of Elution Buffer.Column 2: Gel filtration. Hiload S200 16/60 - 120 ml volume.The gel filtration column was pre-equilibrated with Gel Filtration Buffer. The HisTrap eluant was loaded on the gel filtration column automatically after the HisTrap elution at a flow rate of 1.2 ml/min. Eluted proteins were collected in 1.8 ml fractions. The fractions containing protein were identified on a coomasie blue stained gel.TEV protease digestion: The gel filtration fractions containing DVL2A were pooled and 100 ml of TEV protease solution (~1 mg/ml) was added to 6 mg protein. The digestion was left overnight at 4°C.Rebinding of impurities to Ni-NTA: The protein was mixed with Ni-cellulose resin (0.4 ml, pre-equilibrated into Gel Filtration Buffer) at 4°C for 60 minutes. The resin was spun down and the supernatant was filtered through a 0.2 mM filter and collected.

Extraction

Procedure
Cell Lysis: Two liter-culture pellets were resuspended in lysis buffer. They were passed 4 times through an Emulsiflex C5 high-pressure homogeniser, collecting a final volume of approximately 90 ml. PEI was added to a final concentration of 0.25 % and the cell debris and precipitated DNA were spun down at 45000x g, 90 min (Beckman JA 18 17500 rpm).). The supernatant was filtered through a 0.2 uM filter.
Concentration: The TEV protease cleaved DVL2A was concentrated to 17.4 mg/ml,distributed into 30 ml aliquots and frozen at -80°C.
Ligand
MassSpec: Measured: 10813.3; Expected: 10813.4.
Crystallization: Crystals grew from a 2:1 ratio of protein to precipitant solution (0.2M (NH4) (ac); 0.1M BIS-TRIS pH 5.5; 25% PEG 3350), using the vapour diffusion method.
NMR Spectroscopy:
Data Collection: Data was collected to a resolution of 1.55 Å.
Data Processing: