PDLIM7

PDB:2Q3G

Revision

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Entry Clone Accession:gi|11496885
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SGC Clone Accession:PDLIM7A-c006
Tag:N-terminal, TEV cleavable hexahistidine tag
Host:BL-21(DE3)-R3-Rosetta (A homemade phage resistant version of BL21(DE3) containing the pRARE2 plasmid from Rosetta II (DE3) cells).

Construct

Prelude:
Sequence:

smDSFKVVLEGPAPWGFRLQGGKDFNVPLSISRLTPGGKAAQAGVAVGDWVLSIDGENAGSLTHIEAQNKIRACGERLSLGLSRA*IT*
SL

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 m l 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 1.5 hours. 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.
Column 2: Gel filtration. Hiload S200 16/60 -120 ml volume.

The clarified cell extract was passed through the column at a flow rate of 0.8 ml/min. The column was then washed Binding Buffer until a stable UV baseline was achieved. 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.

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 PDLIM7A (28 mg) were pooled and 400 µl of 1 mg/ml TEV protease solution was added. The digestion was left overnight at 4°C.

Rebinding of impurities to Ni-NTA: The protein was mixed with Ni-NTA 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 0.45 µM filter and collected.

Extraction

Procedure
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 m. 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 0.45 µM syringe filter.
Concentration: The TEV protease cleaved PDLIM7A was concentrated to 37.5 mg/ml (measured by using a Nanodrop), distributed into 30 µl aliquots and frozen at -80°C.
Ligand
MassSpec: Measured: 9292.5;
Expected: 9292.6.
Crystallization: Crystals grew from a 1:1 ratio of protein to precipitant solution (20 % PEG 4K, 10% isopropanol, 0.1 M HEPES pH 7.5), using the vapour diffusion method.
NMR Spectroscopy:
Data Collection: Resolution: 1.1 Å; X-ray source: Synchrotron SLS- SLS-X10.
Data Processing: