PDLIM1

PDB:2PKT

Revision

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Entry Clone Accession:gi|13994151
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SGC Clone Accession:PDLIM1A-c001
Tag:N-terminal, TEV cleavable hexahistidine tag
Host:BL21(DE3)-R3-pRARE2 (A homemade phage resistant version of BL21(DE3) containing the pRARE2 plasmid from Rosetta II (DE3) cells).

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfqsmTTQQIDLQGPGPWGFRLVGGKDFEQPLAISRVTPGSKAALANLCIGDVITAIDGENTSNMTHLE
AQNRIKGCTDNLTLTVARSEHESDL

Vector:pNIC28-Bsa4.

Growth

Medium:TB
Antibiotics:
Procedure: The construct DNA was transformed into homemade chemically competent cells of the expression strain by a standard heat shock procedure.
A number of colonies from the transformation were used to inoculate 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.
10 µl of a thawed glycerol stock was used to inoculate 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 inoculate 2x 1L of TB media (18 ml starter culture into each) containing 50 µg/ml kanamycin. After 4 hours, the temperature was reduced to 22°C. The incubation was continued for 1.5 hours. At OD~3, the cells were induced by 0.5 mM IPTG. The expression was continued overnight (~18 hours). 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 protein was purified using an AktaExpress system.
The clarified cell extract was passed through the column 1 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 PDLIM1A were pooled and 350 µl of TEV protease solution (~1 mg/ml) 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 ml. PEI was added to a final concentration of 0.25 % and the cell debris and precipitated DNA were spun down (38400x g, 90 min). The supernatant was filtered through a 1.2 µM and then a 0.45 µM syringe filter.
Concentration: The TEV protease cleaved PDLIM1A was concentrated to 63 mg/ml (measured using a nanodrop machine), distributed into 30 µl aliquots and frozen at -80°C.
Ligand
MassSpec: Measured: 9723.9; Expected: 9723.8.
Crystallization: Crystals grew from a 1:2 ratio of protein to precipitant solution (40% PEG 300, 0.24 M Ca(ac)2, 0.1M cacodylate pH=6.5), using the vapour diffusion method.
NMR Spectroscopy:
Data Collection: Resolution: 1.5 Å; X-ray source: Synchrotron SLS- SLS-X10.
Data Processing: