Entry Clone Source: MGC

Entry Clone Accession: IMAGE:2959384

SGC Construct ID: PDZK1A-c025

GenBank GI number: gi|21361142

Vector: pNIC28-Bsa4. Details [PDF]; Sequence [FASTA] or [GenBank]

***E.coli* strain:** BL21(DE3)-R3-pRARE2

Tags and additions: N-terminal His-tag with TEV protease cleavage site (Tag sequence in lowercase)
mhhhhhhssgvdlgtenlyfqsMKPKLCR
LAKGENGYGFHLNAIRGLPGSFIKEVQKG
GPADLAGLEDEDVIIEVNGVNVLDEPYEK
VVDRIQSSGKNVTLLVCGKKAQDTV

Growth medium, induction protocol: 10µl of a glycerol stock was inoculated into 5ml of TB medium (supplemented with 50µg/ml Kanamycin) and cultured at 37°C o/n in a shaking incubator (275 rpm). Next day 0.75 ml of o/n culture was used to inoculate 1 litre of TB medium (2x) and grown at 37°C with vigorous shaking (160 rpm) until the culture reaches an OD_{600} of 1.5. Temperature was reduced to 18°C, and cells were induced with IPTG at a concentration of 0.5 mM, and further cultivated for 16 hrs. Cells were harvested by centrifugation at 6500 rpm for 10 min, and the cell pellet was stored at -20°C until further use.

Extraction buffer, extraction method: Lysis buffer: 500 mM NaCl, 5% Glycerol, 50 mM HEPES pH 7.5, 5 mM Imidazole Complete® protease inhibitors (Roche, 1 tbl/50 ml). Frozen cell pellets were thawed and resuspended in a total volume of 30-40 ml of lysis buffer, and disrupted by using Avestin C-5 microfluidizer, and a supernatant containing the target protein was obtained by centrifugation at 21,000 (rpm) for 45 minutes.

Column 1: Ni-Sepharose 6 Fast Flow

Buffers: Lysis buffer: 50 mM HEPES pH 7.5, 500 mM NaCl, 5% Glycerol, 5 mM Imidazole; **Wash buffer:** 50 mM Tris-HCl pH 7.5, 500 mM NaCl, 5% Glycerol, 30 mM Imidazole; **Elution buffer:**50 mM HEPES pH 7.5, 500 mM NaCl, 5% Glycerol, 250 mM Imidazole. **note:** All the buffers contain 0.5mM TCEP.

Procedure: The column was packed with 2 ml of Ni-Sepharose 6 Fast Flow slurry and equilibrated with 15 ml of binding buffer. The supernatant was loaded onto the column and the column was washed with 20 ml of binding buffer and then 20 ml of washing buffer. The protein was eluted with 10 ml of elution buffer.

Column 2: SuperDex S-75 (GE/Amersham)

Buffer: 10 mM HEPES, pH 7.5, 500 mM NaCl, 5 % glycerol, 0.5 mM TCEP.

Procedure: The eluted protein from the Ni-affinity column was loaded on the gel filtration column in GF buffer at 1.0 ml/min on an AKTA Purifier system. Eluted proteins were collected in 1 ml fractions.

Enzymatic treatment: TEV cleaved.

Column 3: Ni-NTA (TEV clean up)

Buffer: 10 mM HEPES, pH 7.5, 500 mM NaCl, 5% glycerol, 0.5 mM TCEP

Procedure: Total 10 mgs of protein was cleaved with 600 ug of TEV protease at 4°C for 48 hours.

TEV clean up: The TEV cleaved protein was applied to a 1 ml Ni-NTA column, already equalibrated with gel filtration buffer (10 mM HEPES, pH 7.5, 500 mM NaCl, 5% glycerol, 0.5 mM TCEP). The flow through form the column was collected. The eluate from the column was monitored by SDS gel analysis.

Concentration: The PDZK1A protein (in buffer; 10 mM HEPES, pH 7.5, 500 mM NaCl, 5% glycerol, 0.5 mM TCEP) was concentrated to 3.0 mg/ml using Vivaspin 5K concentrators and stored at -80°C.
Mass spec characterization: Corresponds to theoretical mass, as determined by ESI-TOF MS.
Crystallization: PDZK1A was crystallized at 20°C using the sitting-drop vapour diffusion method. Diffraction quality crystals were obtained by mixing 100nl of protein solution (3.0 mg/ml) with 50nl reservoir solution (0.1M Na/K-PO_4 pH6.2, 0.20M NaCl and 50% PEG300).
Data Collection: Crystals were flash frozen in liquid nitrogen. Diffraction data were collected to 2.2 Å on beam-line X10SA at the Swiss light source (SLS); **Resolution:** 2.2 Å