

Entry Clone Source: MGC
Entry Clone Accession: IMAGE:4069168
SGC Construct ID: TAX1BP3A-c001
GenBank GI number: gi 11993943
Vector: pNIC28-Bsa4. Details [PDF] ; Sequence [FASTA] or [GenBank]
Tags and additions: N-terminal His-tag with TEV protease cleavage site (*)
Final protein sequence (tag sequence in lowercase):
<pre> mhhhhhhssgvdlgtenlyfqs*mVQRVE IHKLQRQGENLILGFSIGGGIDQDPSQNP SEDKTDKGIVYVTRVSEGGPAEIAGLQIGD KIMQVNGWDMTMVTHDQARKRLTKRSEEV VRLLVTRQSLQKAVQQSMLSETEV </pre>
Host: BL21(DE3)-R3 pRARE2
<p>Growth medium, induction protocol: 10ul of a glycerol stock was inoculated into 5ml of TB medium (supplemented with 50ug/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 OD600 of 1.4. 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.</p>
<p>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.</p>
Column 1: Ni-Sepharose 6 Fast Flow
<p>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.</p>
<p>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.</p>
Column 2: SuperDex 200 16/60 HiLoad (GE/Amersham)
Buffer: 10 mM HEPES, pH 7.5, 500 mM NaCl, 5 % glycerol, 0.5 mM TCEP.
<p>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.</p>
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 degree for 48 hours.

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

Sample Concentration: The protein sample (in buffer; 10 mM HEPES, pH 7.5, 500 mM NaCl, 5% glycerol, 0.5 mM TCEP) was concentrated to 1.5 mg/ml using Vivaspin 10K concentrators and stored at -80C.

Mass spec characterization: Corresponds to theoretical mass, as determined by ESI-TOF MS.

Crystallization: Crystals were grown by vapour diffusion in sitting drops at 4°C. A sitting drop consisting of 150 nl protein and 75 nl well solution was equilibrated against well solution containing 10% PEG 3350, 150 mM Zinc acetate pH 3.8. Crystals were cryo protected in 25% ethylene glycol and flash-cooled in liquid nitrogen.

Data Collection, Resolution: 1.8 Å , **X-ray source:** Synchrotron SLS-X10SA, single wavelength.