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

Tags and additions: Tag sequence: N-terminal His6-tag with the TEV protease recognition site

Final protein sequence (tag sequence in lowercase):
mhhhhhhssgvdlgtenlyfqsmGAGRRE
SEPRPTSARQLDGIRNIVLSNPKKRNTLS
LAMLKSLQSDILHDADSNDLKVIIISAEG
PVFSSGHDLKELTEEQGRDYHAEVFQTCS
KVMMHIRNHPVPVIAMVNGLATAAGCQLV
ASCDIAVASDKSSFATPGVNVGLFCSTPG
VALARAVPRKVALEMLFTGEPISAQEALL
HGLLSKVVPEAELQEETMRIARKIASLSR
PVVSLGKATFYKQLPQDLGTAYYLTSQAM
VDNLALRDGQEGITAFLQKRKPVWSH

Host: BL21(DE3)-R3-pRARE2

Expression protocol: 10 µl plasmid DNA was transformed into 50 µl competent BL-21 (DE3) cells. Colonies were grown in 1 ml TB + 50 mg/ml kanamycin overnight and glycerol stocks were prepared and stored at -80°C. The glycerol stock was used to inoculate 80 ml overnight culture TB supplemented with kanamycin and chloramphenicol at 37°C. 10ml starter culture was used to inoculate six 1L TB culture grown at 37°C until the OD_{600} reached ~1.5. The temperature was lowered to 18°C, 0.5mM IPTG was added to the culture and grown overnight. Cells were harvested by centrifugation at 4000 rpm and stored at -20°C.

Extraction buffer: 50mM HEPES pH 7.5, 500mM NaCl, 5mM Imidazole, 5% glycerol + EDTA-free Complete (1 tablet/50ml).

Procedure: The thawed cellular lysates were supplemented with Igepal CA 630 (Fluka) at a final concentration of 0.05%, and benzonase (Novagen) (25u/10ml of lysate). Cells were broken using a sonicator, followed by centrifugation for 45 min at 20.000rpm at 4°C.

Column 1: Ni-affinity chromatography: HisTrap FF Crude, 5 ml (GE Healthcare).

Buffers: Binding Buffer: 50mM HEPES pH 7.5, 500mM NaCl, 5% glycerol, 0.5mM TCEP, 10mM Imidazole; **Wash Buffer**: 50mM HEPES pH 7.5, 500mM NaCl, 5% glycerol, 0.5mM TCEP, 40mM Imidazole; **Elution Buffer:** 50mM HEPES pH 7.5, 500mM NaCl, 5% glycerol, 0.5mM TCEP, 250mM Imidazole.

Column 2: Size exclusion chromatography HiLoad 16/60 Superdex 75

SEC-Buffers: 10 mM Hepes, pH 7.4, 500 mM NaCl, 5% glycerol,10 mM DTT

Procedure: The cell extract was incubated with Ni-Sepharose (0.250ul of resin/lysate from 1l of culture) during 1h at 4°C with gentle rotation. The resin was centrifuged at 1000g for 5min at 4°C, and washed 4x with 50ml of washing buffer. Resin was loaded on the gravity column and protein was eluted in 4 elution fractions (5ml each). Protein fractions were analysed by SDS-PAGE. Target protein containing fractions were concentrated using Amicon Ultra-15 concentrators with 10kDa cut-off, and purified on a SEC column (Superdex 75) on an Akta Purifier System. Fractions containing protein were analysed by SDS-PAGE.

Mass spec characterization: ESI-MS revealed that the protein had the expected mass of 31238 Da.

Protein concentration: Sample was concentrated to 17.4 mg/ml in SEC buffer using a centricon apparatus with a 10 kDa MWCO.

Crystallization: Crystals were grown by vapor diffusion at 20°C in 150nl sitting drops, consisting of 1:1 mix of purified protein and mother liquor containing 0.20M Na formate, 0.1M BisTris propane pH 8.5, 20.0% PEG 3350 and 10.0% ethylene glycol. Crystals were cryoprotected in 20% ethyleneglycol and flash-frozen in liquid nitrogen.

Data Collection: Resolution: 2.2Å. **X-ray source:** Swiss Light source (SLS), beamline X-10.