FLJ13798 (4AAP) Materials & Methods

Entry Clone Source: MGC

Construct ID: FLJ13798A-c222

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

Final protein sequence (Tag sequence in lowercase):

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mhhhhhhssgvdlgtenlyfq^sMLE
KTVPRLHRPSLQHFREQFLVPGRPVI
LKGVADHWPCMQKWSLEYIQEIAGCR
TVPVEVGSRYTDEEWSQTLMTVNEFI
SKYIVNEPRDVGYLAQHQLFDQIPEL
KQDISIPDYCSLGDGEEEEITINAWF
GPQGTISPLHQDPQQNFLVQVMGRKY
IRLYSPQESGALYPHDTHLLHNTSQV
DVENPDLEKFPKFAKAPFLSCILSPG
EILFIPVKYWHYVRALDLSFSVSFWW
S

^ TEV protease recognition site
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Tags and additions: Cleavable N-terminal His6 tag

Host: BL21(DE3)-R3-pRARE2

Growth Medium & Induction Protocol: Medium: TB supplemented with 50 µg/ml Kanamycin and 34 µg/ml chloramphenicol. 4 liter TB in two 4L flasks were inoculated with 40 ml overnight culture and grown at 37°C until OD_{600} reached 2.5. The temperature was then decreased to 18°C and the protein expression induced with 0.1 mM IPTG over night. The cells were collected by centrifugation (4000 RPM, 30 minutes) and frozen at -80°C.

Extraction buffer, extraction method: The cell pellet was resuspended in a total volume of 200 ml lysis buffer and the cells disrupted by sonication. Nucleic acids and cell debris were removed by adding 0.15% PEI, followed by centrifugation for 60 minutes at 40 000xg. The supernatant was further clarified by filtration (0.20 µm).
Lysis buffer: 50 mM HEPES pH 7.5, 500 mM NaCl, 10 mM Imidazole, 5% glycerol, 3 U/ml of Benzonase, Complete® protease inhibitors (1 tablet/50 ml)

Column 1: Ni-sepharose, HisTrap FF, 5 ml (GE healthcare).

Column 1 Buffers:
Lysis buffer: 50 mM HEPES pH 8.0, 500 mM NaCl, 10 mM Imidazole, 5% glycerol.
Wash buffer: 50 mM HEPES pH 7.5, 500 mM NaCl, 20 mM Imidazole, 5 % glycerol.
Elution buffer: 50 mM HEPES pH 7.5, 500 mM NaCl, 300 mM Imidazole, 5 % glycerol.

Column 2: S200, (GE healthcare)

Column 2 Buffers:
Gel Filtration buffer: 10 mM HEPES pH 7.5, 500 mM NaCl, 5 % glycerol.

Column 2 Procedure: The cell extract was applied onto a 5 ml Ni-Sepharose FF gravity column equilibrated with lysis buffer. The column was subsequently washed with 10 volumes

of lysis buffer followed by 10 wash buffer and protein eluted with 2x3 column volumes of elution buffer. All fractions were collected and analysed by 4-12% SDS-PAGE. The protein was concentrated in amicon 10 K to 5 ml, filtered through a 0.20 µm PVDF filter and applied onto a Superdex S200 column at 1.2 ml/min using an AKTA Xpress system. The eluted proteins were collected in 1.8 ml fractions and analysed by SDS-PAGE.

Column 3: Ni-Sepharose FF (TEV clean up)

Column 3 Buffers:
Wash buffer: 10 mM HEPES, pH 7.5, 500 mM NaCl, 5% glycerol

Column 3 Procedure: The N-terminal histidine-tag was cleaved with 150 µg of TEV protease per 10 mg protein at 4°C for 48 hours. The TEV cleaved protein was applied to a 0.5 ml Ni-sepharose column and the flow through collected. The column was washed with 5 ml buffer and the flow through and the wash fraction were analysed by SDS-PAGE.

Concentration: The protein was concentrated in Amicon 10K to 32 mg/ml. The protein concentration was determined spectrophotometrically using the predicted molar extinction coefficient 53400 (M^{-1} cm^{-1}) and the predicted mass of 27735.7 Da

Mass spec characterization:
Expected: 27735.7
Observed: 27736.3

Crystallization: FLJ13798A was crystallised by vapor diffusion at 20°C from a sitting drop consisting of 100 nl protein (32 mg/ml) and 50 nl 16 % PEG3350, 7 mM $NiCl_2$, 7 mM $MgCl_2$, 0.1 M Hepes pH 7.3. The crystal was transferred to a cryo protectant composed of 33 % glycerol before flash-cooling in liquid nitrogen.

Data Collection:
Resolution: 2.60Å **X-ray source**: Diamond IO2