Entry Clone Source: MGC

Entry Clone Accession: IMAGE:4510603

SGC Construct ID: MGC45594A-c007

GenBank GI number: gi|28557745

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

Amplified construct sequence:

```
CATATGCACCATCATCATCATCATTCTTC
TGGTGTAGATCTGGGTACCGAGAACCTGT
ACTTCCAATCCATGCAGGGCTCCGCCATT
CCCCAAGCCATGCAGAAGCTGGTGGTGAC
CCGGCTGAGCCCCAACTTCCGCGAGGCCG
TCACCCTGAGCCGGGACTGCCCGGTGCCG
CTCCCCGGGGACGGAGACCTCCTCGTCCG
GAACCGATTTGTTGGTGTTAACGCATCTG
ACATCAACTATTCAGCAGGCCGCTATGAC
CCCTCAGTTAAGCCTCCCTTTGACATAGG
TTTCGAAGGCATTGGGGAGGTGGTGGCCC
TAGGCCTCTCTGCTAGTGCCAGATACACA
GTTGGCCAAGCTGTGGCTTACATGGCACC
TGGTTCTTTTGCTGAGTACACAGTTGTGC
CTGCCAGCATTGCAACTCCAGTGCCCTCA
GTGAAACCCGAGTATCTTACCCTGCTGGT
AAGTGGCACCACCGCATACATCAGCCTGA
AAGAGCTCGGAGGACTGTCGGAAGGGAAA
AAAGTTTTGGTGACAGCAGCAGCTGGGGG
AACGGGCCAGTTTGCCATGCAGCTTTCAA
AGAAGGCAAAGTGCCATGTAATTGGAACC
TGCTCTTCTGATGAAAAGTCTGCTTTTCT
GAAATCTCTTGGCTGTGATCGTCCTATCA
ACTATAAAACTGAACCCGTAGGTACCGTC
CTTAAGCAGGAGTACCCTGAAGGTGTCGA
TGTGGTCTATGAATCTGTTGGGGGAGCCA
TGTTTGACTTGGCTGTAGACGCCCTGGCT
ACGAAAGGGCGCTTGATAGTAATAGGGTT
TATCTCTGGCTACCAAACTCCTACTGGCC
TTTCGCCTGTGAAAGCAGGAACATTGCCA
GCCAAACTGCTCAAGAAATCTGCCAGCGT
ACAGGGCTTCTTCCTGAACCATTACCTTT
CTAAGTATCAAGCAGCCATGAGCCACTTG
CTCGAGATGTGTGTGAGCGGAGACCTGGT
TTGTGAGGTGGACCTTGGAGATCTGTCTC
CAGAGGGCAGGTTTACTGGCCTGGAGTCC
ATATTCCGTGCTGTCAATTATATGTACAT
GGGAAAAAACACTGGAAAAATTGTAGTTG
AATTACCTCACTGACAGTAAAGGTGGATA
CGGATCCGAA
```

Tags and additions: N-terminal His-tag with TEV protease cleavage site.

Final protein sequence (tag sequence in lowercase):

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mhhhhhhssgvdlgtenlyfqsMQGSAIP
QAMQKLVVTRLSPNFREAVTLSRDCPVPL
PGDGDLLVRNRFVGVNASDINYSAGRYDP
SVKPPFDIGFEGIGEVVALGLSASARYTV
GQAVAYMAPGSFAEYTVVPASIATPVPSV
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KPEYLTLLVSGTTAYISLKELGGLSEGKK
VLVTAAAGGTGQFAMQLSKKAKCHVIGTC
SSDEKSAFLKSLGCDRPINYKTEPVGTVL
KQEYPEGVDVVYESVGGAMFDLAVDALAT
KGRLIVIGFISGYQTPTGLSPVKAGTLPA
KLLKKSASVQGFFLNHYLSKYQAAMSHLL
EMCVSGDLVCEVDLGDLSPEGRFTGLESI
FRAVNYMYMGKNTGKIVVELPH

^ TEV cleave site

Host: BL21 (DE3)R3-pRARE2 (Phage resistant strain)

Growth medium, induction protocol:10 µl of a glycerol stock was inoculated into 3ml of LB medium (supplemented with Kanamycin, 50 µg/ul) in a 15 ml culture tube and cultured at 37°C overnight in a shaking incubator (275 rpm). Next day 1 ml of overnight culture was used to inoculate 1 litre of LB medium and grown at 37°C with vigorous shaking (180 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 1 mM, and cultivated for 16 hrs. Cells were harvested, centrifuged at 6500 rpm for 10 min, and the pellet was stored at -20°C until further use.

Extraction buffer, extraction method: Thawed cell pellets were dissolved in 30-40 ml of binding buffer (500 mM NaCl, 5%Glycerol, 50 mM HEPES pH 7.5, 5 mM Imidazole). Cells were lysed by sonication (3x 2 minutes) in a 50ml conical tube. After lysis, the cell lysate was centrifuged at 4°C for 45 minutes at 21,000 (rpm).

Column 1: Ni-NTA resin.

Buffers: Wash buffer: 500 mM NaCl, 5% Glycerol, 50 mM Tris-HCl pH 7.5, 30 mM Imidazole;
Elution buffer: 500 mM NaCl, 5% Glycerol, 50 mM HEPES pH 7.5, 250mM Imidazole.

Procedure: The clear supernatant after centrifugation was passed through a Ni-NTA (2.5ml resin) column twice. The column was washed with 50 ml of wash buffer, and protein was eluted with 15 ml of elution buffer.

Column 2: Hiload 16/60 Superdex 200 prep grade 120 ml, GE Healthcare.

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

Procedure: The eluted fractions from the Ni-affinity HisTrap columns were loaded on the gel filtration column in GF buffer at 1.0 ml/min. Eluted proteins were collected in 1 ml fractions.

Mass spectrometry characterization: corresponds to theoretical mass, as determined by ESI-TOF MS.

Protein concentration: Protein was concentrated to 5 mg/ml using Vivaspin 10K concentrators.

Crystallization: Crystals were grown by vapour diffusion in sitting drop at 20°C. Before crystallization setup protein was incubated with 5mM of NADP and 2.5mM of Diclofenac. A sitting drop consisting of 50 nl protein and 50 nl well solution was equilibrated against well solution containing 25% 1,2 propandiol; 10% glycerol; 0.1M Na/K-PO_4 pH 6.2. Crystals were mounted in the presence of 25% glycerol and flash-cooled in liquid nitrogen.

Data Collection: Resolution: 1.9 Å; **X-ray source:** FRE superbright, single wavelength.