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]
***E.coli* strain:** BL21(DE3)-R3-pRARE2
Amplified construct sequence:

```
CATATGCACCATCATCATCATCATTCTTCT
GGTGTAGATCTGGGTACCGAGAACCTGTAC
TTCCAATCCATGCAGGGCTCCGCCATTCCC
CAAGCCATGCAGAAGCTGGTGGTGACCCGG
CTGAGCCCCAACTTCCGCGAGGCCGTCACC
CTGAGCCGGGACTGCCCGGTGCCGCTCCCC
GGGGACGGAGACCTCCTCGTCCGGAACCGA
TTTGTTGGTGTTAACGCATCTGACATCAAC
TATTCAGCAGGCCGCTATGACCCCTCAGTT
AAGCCTCCCTTTGACATAGGTTTCGAAGGC
ATTGGGGAGGTGGTGGCCCTAGGCCTCTCT
GCTAGTGCCAGATACACAGTTGGCCAAGCT
GTGGCTTACATGGCACCTGGTTCTTTTGCT
GAGTACACAGTTGTGCCTGCCAGCATTGCA
ACTCCAGTGCCCTCAGTGAAACCCGAGTAT
CTTACCCTGCTGGTAAGTGGCACCACCGCA
TACATCAGCCTGAAAGAGCTCGGAGGACTG
TCGGAAGGGAAAAAAGTTTTGGTGACAGCA
GCAGCTGGGGGAACGGGCCAGTTTGCCATG
CAGCTTTCAAAGAAGGCAAAGTGCCATGTA
ATTGGAACCTGCTCTTCTGATGAAAAGTCT
GCTTTTCTGAAATCTCTTGGCTGTGATCGT
CCTATCAACTATAAAACTGAACCCGTAGGT
ACCGTCCTTAAGCAGGAGTACCCTGAAGGT
GTCGATGTGGTCTATGAATCTGTTGGGGGA
GCCATGTTTGACTTGGCTGTAGACGCCCTG
GCTACGAAAGGGCGCTTGATAGTAATAGGG
TTTATCTCTGGCTACCAAACTCCTACTGGC
CTTTCGCCTGTGAAAGCAGGAACATTGCCA
GCCAAACTGCTCAAGAAATCTGCCAGCGTA
CAGGGCTTCTTCCTGAACCATTACCTTTCT
AAGTATCAAGCAGCCATGAGCCACTTGCTC
GAGATGTGTGTGAGCGGAGACCTGGTTTGT
GAGGTGGACCTTGGAGATCTGTCTCCAGAG
GGCAGGTTTACTGGCCTGGAGTCCATATTC
CGTGCTGTCAATTATATGTACATGGGAAAA
AACACTGGAAAAATTGTAGTTGAATTACCT
CACTGACAGTAAAGGTGGATACGGATCCGA
A
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Tags and additions: N-terminal His-tag with TEV protease cleavage site
Final protein sequence (tag sequence in lowercase):

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mhhhhhhssgvdlgtenlyfqsmQGSAIPQ
AMQKLVVTRLSPNFREAVTLSRDCPVPLPG
DGDLLVRNRFVGVNASDINYSAGRYDPSVK
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PPFDIGFEGIGEVVALGLSASARYTVGQAV
AYMAPGSFAEYTVVPASIATPVPSVKPEYL
TLLVSGTTAYISLKELGGLSEGKKVLVTAA
AGGTGQFAMQLSKKAKCHVIGTCSSDEKSA
FLKSLGCDRPINYKTEPVGTVLKQEYPEGV
DVVYESVGGAMFDLAVDALATKGRLIVIGF
ISGYQTPTGLSPVKAGTLPAKLLKKSASVQ
GFFLNHYLSKYQAAMSHLLEMCVSGDLVCE
VDLGDLSPEGRFTGLESIFRAVNYMYMGKN
TGKIVVELPH

Host: E.coli strain BL21DE3- R3

Growth medium, induction protocol: 10ul of a glycerol stock was inoculated into 3ml of LB medium (supplemented with Kanamycin, 50ug/ul) in a 15 ml culture tube and cultured at 37°C o/n in a shaking incubator (275 rpm). Next day 1 ml of o/n 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

Column 1 Buffers:
Binding buffer: 500 mM NaCl, 5%Glycerol, 50 mM HEPES pH 7.5, 5 mM Imidazole
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

Column 1 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 (500 mM NaCl, 5% Glycerol, 50 mM
Tris-HCl pH 7.5, 30 mM Imidazole), and protein was eluted with 15 ml of elution buffer (500 mM NaCl, 5% Glycerol, 50 mM HEPES pH 7.5, 250mM Imidazole).

Column 2: Hiload 16/60 Superdex 200 prep grade 120 ml (GE Healthcare)

Column 2 Buffer: 10 mM HEPES, pH 7.5, 500 mM NaCl, 5 % glycerol, 0.5 mM TCEP.

Column 2 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

Concentration: 5 mg/ml using Vivaspin 10K concentrators

Enzymatic treatment: none

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

Crystallization: Crystals were grown by vapour diffusion in sitting drop at 20°C. Before crystallization setup protein was incubated with 5mM of NADP and 0.4 mM of Fenoprofen. A sitting drop consisting of 100 nl protein and 50 nl well solution was equilibrated against well solution containing 0.5M Na-malonate pH 7.0, 0.25% w/v jeffamine-ED-2001 pH 7.0, 0.1M HEPES pH 8.0. Crystals were cryo-protected in the presence of 25% ethylene glycol and flash-cooled in liquid nitrogen.

Data Collection: Resolution: 1.6 Å **, X-ray source**: Diamond Light Source, beamline I03, single wavelength.