Entry Clone Source: Origene

Entry Clone Accession: NM_015061 variant

SGC Construct ID: JMJD2CA-c705

GenBank GI number: gi|24307987

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

Amplified construct sequence:
CATATGCACCATCATCATCATCATTCTTCT
GGTGTAGATCTGGGTACCGAGAACCTGTAC
TTCCAATCCATGTGCGAGAAGGTCATTTCC
GTGGGTCAAACGGTCATCACGAAGCATCGG
AACACCCGGTATTACAGTTGCAGAGTGATG
GCTGTGACATCGCAGACCTTCTATGAGGTC
ATGTTTGATGATGGCTCCTTTAGCAGAGAC
ACATTTCCTGAGGATATCGTGAGCCGAGAC
TGTCTGAAGCTGGGCCCACCTGCTGAGGGA
GAAGTCGTCCAAGTCAAGTGGCCCGATGGC
AAACTCTATGGAGCAAAATATTTTGGATCA
AATATTGCCCACATGTACCAGGTTGAGTTT
GAAGATGGATCCCAGATAGCAATGAAGAGA
GAGGACATCTACACTTTAGATGAAGAGTTA
CCCAAGAGAGTGAAATGACAGTAAAGGTGG
ATACGGATCCGAA

Final protein sequence:
SMCEKVISVGQTVITKHRNTRYYSCRVMAV
TSQTFYEVMFDDGSFSRDTFPEDIVSRDCL
KLGPPAEGEVVQVKWPDGKLYGAKYFGSNI
AHMYQVEFEDGSQIAMKREDIYTLDEELPK
R

Tags and additions: N-terminal, TEV cleavable hexahistidine tag

Host: *Trichoplusia Ni* (High five)

Expression strain: BL-21(DE3)-R3-Rosetta (A homemade phage resistant version of BL21(DE3) containing the pRARE2 plasmid from Rosetta II (DE3) cells).
Transformation: The construct DNA was transformed into competent cells of the expression strain by a standard heat shock procedure.
Glycerol stock preparation: A number of colonies were used to inoculate 1 ml of LB media containing 50 µg/ml kanamycin and 34 µg/ml chloramphenicol, which was incubated in a 37°C shaker overnight. The next day glycerol stocks were prepared from this overnight culture.
Expression: 10 µl of a glycerol stock was used to inoculate 120 ml of LB containing 50 µg/ml kanamycin and 34 µg/ml chloramphenicol, which was incubated at 37°C overnight. 15ml starter culture was used per litre TB, containing 50 µg/ml kanamycin. The culture was incubated at 37°C until OD~1.5, when the temperature of the incubator was reduced to 18°C. At 18°C, expression was induced with 0.1 mM IPTG and the culture continued o/n.
Cell harvest: Cells were pelleted at 6238x g for 15 min at 4°C, and stored at -80°C. The yield was 12g cells / litre culture.

Cell Lysis: Lysis Buffer: 50mM HEPES pH 7.4, 500mM NaCl, 5% glycerol, 10 mM Imidazole pH 7, 1tbl Complete (mixture of proteinase inhibitors -EDTA)**.**
The pellets were resuspended in lysis buffer. They were passed 4 times through an Emulsiflex C5 high-pressure homogeniser, collecting a final volume of approximately 25 ml/ litre culture. Cell debris and DNA were spun down at 45000x g, 60 min (Beckman JA 18 17500 rpm). The supernatant was collected to which Benzonase was added with a 30-60 min incubation on ice.

Buffers:
Wash Buffer: 50 mM Hepes pH 7.4, 500 mM NaCl, 5% Glycerol, 30 mM Imidazole pH 7.4
Elution Buffer:50 mM Hepes pH 7.4, 500 mM NaCl, 5% Glycerol, 250 mM Imidazole pH 7.4
Gel Filtration Buffer: 50 mM Hepes pH 7.4, 500 mM NaCl , 5% glycerol

Purification: Purification was performed in an Akta Express system (GE Healthcare) with an automated program for Ni-affinity (HisTrap FF) and gel filtration (HiLoad 16/60 S75) chromatography steps.
Step 1: Ni-affinity, HisTrap Crude FF, 5 ml. The clarified cell extract was loaded on the column at 5 ml/min on the ÄKTA-express system. The column was washed with 15 cv of binding buffer, 10 cv of wash buffer, and then eluted with elution buffer at 4 ml/min. The eluted peak of A_{280} was automatically collected.
Step 2: Gel filtration, Hiload 16/60 Superdex S75 prep grade, 120 ml**.** The eluted fraction from the Ni-affinity column was loaded on the gel filtration column, pre-equilibrated in GF buffer, at 1.2 ml/min. Eluted proteins were collected in 2 ml fractions and analyzed on SDS-PAGE.

TEV protease digestion: Peak fractions from the gel filtration containing JMJD2CA were pooled and TEV protease was added at a molar ratio of 1:30. The digestion was left overnight at 4 °C. SDS-PAGE confirmed complete digestion. His-TEV and contaminating proteins were removed by binding to Ni resin, pre-equilibrated in GF buffer. The flow through containing TEV-cleaved protein was collected and concentrated using a centricon centrifugal device with a 10kDa MWCO. The concentrated protein was centrifuged 20 min 14000rpm, 4°C. The final concentration of protein was 20 mg/ml and yield 3.3 mg/litre culture. The protein was flash frozen and stored at -80°C in 70µl aliquots.

Mass spectrometry characterization: Measured: 13908.9; Expected: 13907.7. MSMS confirmed that the protein was the correct target.

Crystallisation: Crystals were grown at 4°C by vapour diffusion in sitting drops mixing protein (20mg/ml) and precipitant solution (0.2M $(NH_4)_2SO_4$; 0.1M TRIS pH 8.5; 25% PEG 3350) in a 1:2 ratio. Crystals were cryo-protected in 25% glycerol and flash frozen in liquid nitrogen.

Data collection: Data was collected to a resolution of 1.65 Å at Diamond beamline IO3.