GI number: gi|4885251

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

Amplified construct sequence:

ATGCACCATCATCATCATCATTCTTCTGGT
GTAGATCTGGGTACCGAGAACCTGTACTTC
CAATCCATGGCGGGTCCAGGCTCCCTGGGC
GGTGCTTCCGGCCGCGACCAGAGTGACTTC
GTGGGGCAGACGGTGGAACTGGGCGAGCTG
CGGCTGCGGGTGCGGCGGGTCCTGGCCGAA
GGAGGGTTTGCATTTGTGTATGAAGCTCAA
GATGTGGGGAGTGGCAGAGAGTATGCATTA
AAGAGGCTATTATCCAATGAAGAGGAAAAG
AACAGAGCCATCATTCAAGAAGTTTGCTTC
ATGAAAAAGCTTTCCGGCCACCCGAACATT
GTCCAGTTTTGTTCTGCAGCGTCTATAGGA
AAAGAGGAGTCAGACACGGGGCAGGCTGAG
TTCCTCTTGCTCACAGAGCTCTGTAAAGGG
CAGCTGGTGGAATTTTTGAAGAAAATGGAA
TCTCGAGGCCCCCTTTCGTGCGACACGGTT
CTGAAGATCTTCTACCAGACGTGCCGCGCC
GTGCAGCACATGCACCGGCAGAAGCCGCCC
ATCATCCACAGGGACCTCAAGGTTGAGAAC
TTGTTGCTTAGTAACCAAGGGACCATTAAG
CTGTGTGACTTTGGCAGTGCCACGACCATC
TCGCACTACCCTGACTACAGCTGGAGCGCC
CAGAGGCGAGCCCTGGTGGAGGAAGAGATC
ACGAGGAATACAACACCAATGTATAGAACA
CCAGAAATCATAGACTTGTATTCCAACTTC
CCGATCGGCGAGAAGCAGGATATCTGGGCC
CTGGGCTGCATCTTGTACCTGCTGTGCTTC
CGGCAGCACCCTTTTGAGGATGGAGCGAAA
CTTCGAATAGTCAATGGGAAGTACTCGATC
CCCCCGCACGACACGCAGTACACGGTCTTC
CACAGCCTCATCCGCGCCATGCTGCAGGTG
AACCCGGAGGAGCGGCTGTCCATCGCCGAG
GTGGTGCACCAGCTGCAGGAGATCGCGGCC
GCCCGCAACGTGAACCCCAAGTCTCCCATC
ACAGAGCTCCTGGAGCAGAATGGAGGCTAC
GGGAGCGCCACACTGTCCCGAGGGCCATGA

Expressed sequence:

mhhhhhhssgvdlgtenlyfq*smAGPGSLGGAS
GRDQSDFVGQTVELGELRLRVRRVLA
EGGFAFVYEAQDVGSGREYALKRLLS
NEEEKNRAIIQEVCFMKKLSGHPNIV
QFCSAASIGKEESDTGQAEFLLLTELC
KGQLVEFLKKMESRGPLSCDTVLKIF
YQTCRAVQHMHRQKPPIIHRDLKVE
NLLLSNQGTIKLCDFGSATTISHYPD
YSWSAQRRALVEEEITRNTTPMYRT
PEIIDLYSNFPIGEKQDIWALGCILYL
LCFRQHPFEDGAKLRIVNGKYSIPPH

DTQYTVFHSLIRAMLQVNPEERLSIA
EVVHQLQEIAAARNVNPKSPITELLE
QNGGYGSATLSRGP

* TEV cleavage site; the vector-derived sequences are in lowercase.

Tags and additions: Cleavable N-terminal His6 tag.

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

Growth medium, induction protocol: 50Âµl of LB culture started from glycerol stocks was added to 50mls fresh Minimal Pink Medium (MCSG) containing 1.5mg kanamycin, 7.5mg amplicillin, 0.05mg vitamin B1, and 0.135 mg vitamin B12, M9 salts, non-inhibitory amino acids, metal supplements, glucose and glycerol as described in paper (Donnelly, MI *et al*, 2006). Cultures were grown over-night at 37Â°C (150rpm). The 50ml over-night culture was divided equally in to 2 x 1L of freshly prepared Minimal Pink Medium. Cultures were grown at 37Â°C (180rpm) until the OD_{600} reached ~1.0. Next, 90mg of seleno-methionine and 150mg each of inhibitory amino acids (VILKTF) was added and culture was transferred to a pre-cooled 4Â°C incubator (180rpm) for 1 hour. Next, protein expression was induced using 0.5 mM IPTG.Ã, Cultures were switched to an 18Â°C incubator and grown over-night. The cells were collected by centrifugation and the pellet re-suspended in lysis buffer and frozen in -80Â°C.
Lysis buffer: 50 mM HEPES pH 8.0; 500 mM NaCl; 20 mM Imidazole, 5 % Glycerol, 10mM Beta-mercaptoethanol.
Extraction buffer, extraction method: Frozen cell pellets were thawed and fresh lysozyme was added at a final concentration of 1mg/mL to the 40mL of lysate. Cells were further lysed using sonication (Misonix 3000). The lysate was centrifuged (RC5C-Plus centrifuge, Sorval SS-34 rotor) at 13,000 rpm for 45 minutes and the supernatant was filtered through a 0.45Âµm in line filter (Pall) prior to loading on nickel columns (GE HS) using Ã„KTA Xpress.

Buffers for Immobilized Metal Affinity Chromatography I (IMAC I) Using the Ã„KTA Xpress (General Electric Health Systems (GE HS)):
Desalting buffer: 50 mM HEPES pH 8.0, 500 mM NaCl, 5% Glycerol, 10mM Beta-mercaptoethanol.
Lysis buffer: 50 mM HEPES pH 8.0, 500 mM NaCl, 20 mM Imidazole, 5% Glycerol, 10mM Beta-mercaptoethanol.
Elution buffer: 50 mM HEPES pH 8.0, 500 mM NaCl, 250 mM Imidazole , 5% Glycerol, 10mM Beta-mercaptoethanol.

IMAC I Procedure and TEV Protease Cleavage: IMAC-I using a 5-ml HiTrap Chelating HP column charged with Ni^{+2} ions and buffer-exchange chromatography on a HiPrep 26/10 desalting column (both GE Health Systems) were performed using Ã„KTA Xpress (GE HS). The His6-tag was cleaved using the recombinant TEV protease expressed from the vector pRK5084 (a gift from Dr. D. Waugh, NCI). The TEV protease was added to the target protein in a ratio of 1:50 and the solution was incubated at 4Â°C for 48 hours.

Buffers for Immobilized Metal Affinity Chromatography II (IMAC II) Using the Ã„KTA Xpress (GE HS):
Lysis buffer: 50 mM HEPES pH 8.0, 500 mM NaCl, 20 mM Imidazole, 5% Glycerol, 10mM Beta-mercaptoethanol.
Elution buffer: 50 mM HEPES pH 8.0, 500 mM NaCl, 250 mM Imidazole, 5% Glycerol, 10mM Beta-mercaptoethanol.

IMAC II Procedure: The proteins with His6-tag removed were purified IMAC-II using a 5-ml HiTrap Chelating HP column (GE HS) charged with Ni^{+2} ions. Protein was eluted and collected at Imidazole concentrations of 20 mM and 35 mM.

Mass spectrometry characterization: Not determined
Protein concentration: Protein was buffer exchanged in to crystallization buffer (20 mM HEPES pH 8.0, 250 mM NaCl, and 2 mM dithiothreitol (DTT)) during concentration and concentrated to 16 mg/ml using an Amicon Ultra 15 - 3 kDa cut-off concentrator.
Crystallisation: Protein was proteolyzed with 1mg/ml chymotrypsin (1:80 v/v) for 2 hours on ice prior to crystallization set-up. Crystals were grown at 16Â°C in 400 nL sitting drops from a 1:1 ratio of reservoir solution (1.0M succinic acid pH 7.0, 0.1M Bis-Tris Propane pH 7.0) and proteolyzed protein.
Data Collection: Crystals were cryo-protected using the reservoir well solution supplemented with 25% glycerol and flash frozen in liquid nitrogen. **X-ray source:** Diffraction data were collected from a single crystal on APS beamline 19ID at a single wavelength of 0.9794 Ã…. **Phasing:** The structure was by solved via SAD phasing using Se anomalous signal.Ã, The resolution is 2.1 Ã… and is deposited to PDB (3LL6).
Reference: Donnelly, M.I., Zhou, M., Millard, C.S., Stols, L., Eschenfeldt, W.H., Collart, F.R. and Joachimiak, A. (2006) Protein Expression and Purification 47:446-454.