Entry Clone Source: MGC

Entry Clone Accession: IMAGE:2819330

SGC Construct ID: PGDA-c000

GenBank GI number: gi|40068518

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

Tags and additions: N-terminal, TEV cleavable hexahistidine tag. Tag sequence:
mhhhhhhssgvdlgtenlyfq(*)sm

Host:*E. coli* BL21(DE3)-R3

Sequence (tag sequence in lowercase):
mhhhhhhssgvdlgtenlyfqsmAQADIA
LIGLAVMGQNLILNMNDHGFVVCAFNRTV
SKVDDFLANEAKGTKVVGAQSLKEMVSKL
KKPRRIILLVKAGQAVDDFIEKLVPLLDT
GDIIIDGGNSEYRDTTRRCRDLKAKGILF
VGSGVSGGEEGARYGPSLMPGGNKEAWPH
IKTIFQGIAAKVGTGEPCCDWVGDEGAGH
FVKMVHNGIEYGDMQLICEAYHLMKDVLG
MAQDEMAQAFEDWNKTELDSFLIEITANI
LKFQDTDGKHLLPKIRDSAGQKGTGKWTA
ISALEYGVPVTLIGEAVFARCLSSLKDER
IQASKKLKGPQKFQFDGDKKSFLEDIRKA
LYASKIISYAQGFMLLRQAATEFGWTLNY
GGIALMWRGGCIIRSVFLGKIKDAFDRNP
ELQNLLLDDFFKSAVENCQDSWRRAVSTG
VQAGIPMPCFTTALSFYDGYRHEMLPASL
IQAQRDYFGAHTYELLAKPGQFIHTNWTG
HGGTVSSSSYNA

Expression: 10µl of BL21(DE3)-R3 glycerol stock were inoculated into 100ml of TB with 50µg/ml of kanamycin and 34µg/ml chloramphenicol and grown overnight at 37°C, 200rpm. 10ml of overnight culture were added to 1L of TB with 50ug/ml kanamycin and incubated at 37°C, 160rpm. After the OD_{600} reached 1.0, the temperature was dropped to 18°C and 100ul of 1M IPTG was added to the final concentration of ~0.1mM. The culture was then incubated with shaking overnight at 18°C, 160rpm. The following morning the 6L culture was harvested and centrifuged for 15min at 4000rpm. Supernatant was discarded and cell pellets were resuspended in 80ml of a lysis buffer and frozen at -80°C.

Extraction: Lysis buffer: 50mM HEPES pH 7.5, 500mM NaCl, 20mM Imidazole, 5% glycerol + EDTA-free Complete (1 tablet/50ml). The thawed cells were broken by 5 passes at 16.000 psi through a high pressure homogeniser followed by centrifugation for 45 min at 15.000rpm.

Purification:
Column 1: Ni-affinity, His-Trap, 1 ml (Amersham)
Column 2: Superdex 200, HiPrep 16/60 (Amersham)

Buffers: Start buffer: 50mM HEPES pH 7.5, 500mM NaCl, 20mM Imidazole, 5% glycerol, EDTA-free Complete, 0.5mM TCEP; **Washing buffer:** 50mM HEPES pH 7.5, 500mM NaCl, 40mM Imidazole, 5% glycerol, EDTA-free Complete, 0.5mM TCEP; **Elution buffer:** 50mM HEPES pH 7.5, 500mM NaCl, 5% glycerol, 250mM Imidazole, 0.5mM TCEP; **GF buffer:** 10mM HEPES pH 7.5, 500mM NaCl, 5% glycerol, 0.5mM TCEP.

Procedure: The cell extract was loaded on the AKTA Express system The extinction at 280nm was monitored and fractions were collected and analyzed by SDS-PAGE. Positive fractions were pooled and characterised by mass spectrometry.

Concentration: Using Amicon Ultra-15 concentrators with 10 kDa cutoff and concentrated to 16mg/ml. Concentrations were determined from the absorbance at 280 nm using NanoDrop.
Mass spectrometry characterization: Calculated mass of the construct was 55693. The exact mass was confirmed by mass spectrometry.
Crystallisation: Crystals were grown by vapor diffusion at 4°C in 150nl sitting drops. 5mM NADPH was added to the protein aliquot prior to crystallisation. The drops were prepared by mixing 50nl of protein solution and 100nl of precipitant consisting of 0.2M Na_2SO_4, 20% w/v PEG 3350 and 10% ethylene glycol. Crystals were transferred to a cryo-protectant consisting of 10% ethylene glycol and 90% well solution before flash-cooling in liquid nitrogen.
Data Collection: Resolution: 2.53Å; **X-ray source:** SLS beam X10SA.