SNX33A (4AKV) Materials & Methods

Entry Clone Source: MGC

Entry Clone Accession: gi|4869639

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

DNA sequence:

```
CATATGCACCATCATCATCATCATTC
TTCTGGTGTAGATCTGGGTACCGAGA
ACCTGTACTTCCAATCCATGTACTCC
ATTGAAATGGGCCCTCGTGGCCCCCA
GTGGAAGGCCAATCCCCACCCATTTG
CCTGCTCTGTGGAGGACCCCACAAAA
CAGACCAAATTCAAGGGCATCAAAAG
CTACATCTCCTACAAGCTCACACCCA
CCCATGCTGCCTCACCCGTCTACCGG
CGCTACAAACACTTTGACTGGCTCTA
TAACCGCCTGCTACACAAGTTCACTG
TCATCTCGGTGCCCCACCTGCCTGAG
AAGCAGGCCACTGGCCGCTTCGAGGA
GGACTTCATCGAAAAGCGGAAGCGGA
GACTCATCCTCTGGATGGACCACATG
ACCAGCCACCCTGTGCTCTCCCAGTA
CGAAGGCTTCCAGCATTTCCTCAGCT
GCCTGGATGACAAGCAGTGGAAGATG
GGCAAACGCCGGGCGGAGAAGGATGA
GATGGTGGGTGCCAGCTTCCTGCTCA
CCTTCCAGATCCCCACCGAGCACCAG
GACTTGCAGGACGTGGAAGATCGCGT
GGACACTTTCAAGGCCTTCAGTAAGA
AGATGGACGACAGCGTCCTGCAGCTC
AGCACTGTGGCATCAGAGCTGGTGCG
TAAACATGTGGGGGGCTTCCGCAAGG
AATTCCAGAAGCTGGGCAGTGCCTTC
CAGGCCATCAGTCATTCCTTCCAGAT
GGACCCCCCCTTTTGCTCTGAGGCCC
TCAACAGTGCCATTTCTCACACGGGC
CGTACCTATGAAGCCATCGGGGAGAT
GTTTGCTGAGCAGCCCAAGAATGACC
TCTTCCAGATGCTGGACACACTGTCT
CTCTACCAGGGCCTGCTCTCCAACTT
CCCTGACATCATCCATCTACAAAAAG
GCGCCTTCGCCAAGGTGAAGGAGAGC
CAACGCATGAGTGACGAGGGCCGCAT
GGTGCAGGACGAGGCAGACGGCATTC
GCAGGCGCTGCCGCGTGGTGGGTTTC
GCCCTGCAGGCCGAGATGAACCACTT
CCACCAGCGCCGTGAGCTCGACTTCA
AGCACATGATGCAGAACTACTTGCGC
CAGCAGATCCTCTTCTACCAGCGGGT
```

GGGCCAGCAGCTGGAGAAGACCCTGC
GCATGTATGACAACCTCTGACAGTAA
AGGTGGATACGGATCCGAA

Final protein sequence (Tag sequence in lowercase):
mhhhhhhssgvdlgtenlyfq^smYS
IEMGPRGPQWKANPHPFACSVEDPTK
QTKFKGIKSYISYKLTPTHAASPVYR
RYKHFDWLYNRLLHKFTVISVPHLPE
KQATGRFEEDFIEKRKRRLILWMDHM
TSHPVLSQYEGFQHFLSCLDDKQWKM
GKRRAEKDEMVGASFLLTFQIPTEHQ
DLQDVEDRVDTFKAFSKKMDDSVLQL
STVASELVRKHVGGFRKEFQKLGSAF
QAISHSFQMDPPFCSEALNSAISHTG
RTYEAIGEMFAEQPKNDLFQMLDTLS
LYQGLLSNFPDIIHLQKGAFAKVKES
QRMSDEGRMVQDEADGIRRRCRVVGF
ALQAEMNHFHQRRELDFKHMMQNYLR
QQILFYQRVGQQLEKTLRMYDNL

^ TEV protease recognition site

Tags and additions: Cleavable N-terminal His6 tag

Host: BL21(DE3)-R3-pRARE2

Growth Medium & Induction Protocol: The construct DNA was transformed into competent cells of the expression strain by a standard heat shock procedure. One colony from the transformation was used to inoculate 1 ml of TB media containing 50 µg/ml kanamycin and 34 µg/ml chloramphenicol, which was placed in a 37°C shaker overnight. The next day glycerol stocks were prepared from this overnight culture. A glycerol stock was used to inoculate 50 ml of TB media containing 50 µg/ml kanamycin and 34 µg/ml chloramphenicol, which was placed in a 37°C shaker overnight. The next day this starter culture was used to inoculate 6L of TB media (7.5 ml starter culture used per 1L) containing 50 µg/ml kanamycin. When the OD_{600} reached approximately 1.4 the temperature was reduced to 18°C and after a further 30 minutes the cells were induced by the addition of 0.1 mM IPTG. The expression was continued overnight

Extraction buffer, extraction method: Cell pellets were dissolved in approximately 50ml lysis buffer and broken by homogenization by 5 passes at 12,000 psi. The cell debris was pelleted at 40,000 x g and the supernatant used for further purification.
Lysis buffer: 50 mM Hepes pH 7.4, 500 mM NaCl, 5% Glycerol, 10 mM Imidazole pH 7.4, 0.5 mM TCEP, 1 tablet per 50 ml protease inhibitor cocktail EDTA-free (Roche)

Column 1: Ni-NTA (2.5 ml volume in a gravity-flow column).

Column 1 Buffers:
Binding buffer: 50 mM Hepes pH 7.4, 500 mM NaCl, 5% Glycerol, 10 mM Imidazole pH 7.4, 0.5 mM TCEP.
Wash buffer: 50 mM Hepes pH 7.4, 500 mM NaCl, 5% Glycerol, 40 mM Imidazole pH 7.4, 0.5 mM TCEP.
Elution buffer: 50 mM Hepes pH 7.4, 500 mM NaCl, 5% Glycerol, 250 mM Imidazole pH 7.4, 0.5 mM TCEP.

Column 1 Procedure: The clarified cell extract was incubated with 2.5 ml of Ni-NTA pre-equilibrated with lysis buffer for 1 hour at 4°C with rotation after which it was passed through a glass column. The column was then washed with Binding Buffer (50 ml) and Wash Buffer (50 ml). The protein was eluted with 25 ml of Elution Buffer in 5 x 5 ml fractions.

Column 2: Superdex 200 16/60 Gel Filtration

Column 2 Buffers:
Gel Filtration buffer: 10 mM Hepes pH 7.4, 500 mM NaCl, 0.5 mM TCEP, 5% Glycerol.

Column 2 Procedure: The first two elution buffer fractions from column 1 were pooled and concentrated to 2 ml with a 10 kDa mwco spin concentrator and injected into an s200 16/60 column (pre-equilibrated in GF Buffer) at 1.0 ml/min. 1.0 ml fractions were collected. The protein eluted at between 70 ml and 80 ml volume.

Concentration: Protein fractions between 70 and 80 ml elution volume from the gel filtration were pooled and concentrated to 24 mg/ml using a 10 kDa mwco concentrator

Mass spec characterization:
Expected: 45300.8
Observed: 45300.2

Crystallization: Crystals were grown by vapour diffusion in sitting drop at 20°C. A sitting drop consisting of 100 nl protein and 50 nl well solution was equilibrated against well solution containing 0.1 M bis-tris pH 6.0, 0.15 M lithium sulfate and 15% PEG 3350. Crystals were mounted in the presence of 25% (v/v) ethylene glycol and flash-cooled in liquid nitrogen.

Data Collection:Data Collection:
Resolution: 2.70Å **X-ray source**: Diamond IO3