

SND1

PDB:3OMC

Revision

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Entry Clone Accession:BC017180

Entry Clone Source:AU47-C12

SGC Clone Accession:P100_4; plate JMC01I:F9

Tag:N-terminal tag: MGSSHHHHHHSSRENLYFQG

Host:*E. coli* BL21(DE3)-V2R-pRARE2

Construct

Prelude:

Sequence:

AKQKKEKVWAHYEEQPVEEVMPVLEEKERSASYKPVFVTEITDDLHFYVQDVETGTQLEKLMENMRNDIASHPPVEGSYAPRRGEFC
IAKFVDGEWYRARVEKVESPAKIHVFYIDYGNREVLPSRLGTLSPAFSTRVLPQAQTEYAFAFIQVPQDDARTDAVDSVVRDIQN
TQCLLNVEHLSAGCPHVTLQFADSKGDVGLGLVKEGLVMVEVRKEKQFQKVITEYLNAQESAKSARLNLWRYGDFRADDADDFGYSR

Vector:pET28-MHL

Growth

Medium:TB media containing 50 µg/mL kanamycin and 30 µg/mL chloramphenicol

Antibiotics:

Procedure:A fresh transformation was used to inoculate 60 mL LB media containing 50 µg/mL kanamycin and 30 µg/mL chloramphenicol. The culture was grown overnight at 37°C with shaking. The next day this starter culture was used to inoculate 2L of TB growth medium containing 50 µg/mL kanamycin and 30 µg/mL chloramphenicol. The culture was grown in LEX at 37°C to OD₆₀₀ of 2.5. The temperature was reduced to 14µC and IPTG-based induction (1mM) was carried out according to the manufacturer's protocol. The culture was incubated for a further 18 hours before harvesting the cells. Cells were harvested by centrifugation and pellets were stored at -80°C.

Purification

Procedure

Column 1: Affinity purification, open Ni-NTA column

Procedure: The supernatant was incubated with 6mL of 50% slurry Ni-NTA beads (buffer is changed to lysis buffer prior to use) by rocking. After 1 hour incubation at 4oC, the bead mixture

was transferred to an empty column and washed with wash buffer, then using 15ml elution buffer elute. Parts of eluted sample were treated with V8 and TEV which was using for crystallization.

Column 2: Size Exclusion, HiLoad 16/60 Superdex 75 Prep Grade

Procedure: The elution from the NiNTA column and treated protein were concentrated using 15 mL concentrators with a 10K Da molecular weight cut-off (Amicon Ultra-15, Millipore). The concentrated protein was loaded onto the size exclusion column at a flow rate of 1 mL/min, and 2 mL fractions were collected. The fractions containing protein were identified on an SDS-PAGE gel. Pool the fractions together and concentrated it using the 15 mL concentrators with a 10K Da molecular weight cut-off (Amicon Ultra-15, Millipore).

Extraction

Procedure

Prior to purification, the cell pellet was resuspended in lysis buffer. Cells were disrupted by sonication (100 watts, 10 minutes total time using 10 second pulses followed by 10 second rest) on ice and samples were centrifuged for 60 min at 16000RPM.

Concentration: The final concentration was 15 mg/ml. The protein yield was approximately 40 mg per liter of bacterial culture.

Ligand

MassSpec:

Crystallization: Purified protein treated with V8 and TEV protease was mixed with PIWIL1 peptides by directly adding a three-fold or five-fold molar excess of peptide to the protein solution. The crystals of SND1 appeared in a condition containing 30% PEG 1500 at 18°C overnight by the sitting drop vapor diffusion method. Prior to the data collection, the SND1 crystals were freshly soaked in the mother liquor containing 15% glycerol and flash-frozen in liquid nitrogen.

NMR Spectroscopy:

Data Collection:

Data Processing: