TargetÂ ID: SNX14BÂ
EntryÂ CloneÂ ID: SNX14B-s001
AlleleÂ ID: SNX14B-a101
ConstructÂ ID SNX14B-c101
CloneÂ ID SNX14B-k101
ExpressionÂ ID SNX14B-e110
PurificationÂ ID: SNX14B-p014
EntryÂ cloneÂ source: MGC
EntryÂ cloneÂ accession: BC005110 Â
Vector: pNIC28-Bsa4
E.coliÂ strain: BL21(DE3)-R3-pRARE2Â
TagsÂ andÂ additions: N-terminal,Â TEVÂ proteaseÂ cleavableÂ hexahistidineÂ tag

CodingÂ DNAÂ sequence

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ATGGTGCCCTGGGTGCGGACGATGGG
GCAGAAGCTGAAGCAGCGGCTGCGAC
TGGACGTGGGACGCGAGATCTGCCGC
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GCTCTGTCTCAGCGCCGCCTCCCTGC
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GAAGTTACCTCTGTGACATCTTGGAT
GTAA

FinalÂ proteinÂ sequence

mhhhhhhssgvdlgtenlyfq*sMTP
RNLAAWKISIPYVDFFEDPSSERKEK
KERIPVFCIDVERNDRRAVGHEPEHW
SVYRRYLEFYVLESKLTEFHGAFPDA
QLPSKRIIGPKNYEFLKSKREEFQEY
LQKLLQHPELSNSQLLADFLSPNGGE
TQFLDKILPDVNL

MHHHHHHSSGVDLGTENLYFQ*SMÂ isÂ theÂ purificationÂ tagÂ (lower case) plusÂ TEVÂ proteaseÂ recognitionÂ siteÂ (*).

Expression

ExpressionÂ strain

BL21(DE3)-R3-pRARE2Â

AÂ glycerolÂ stockÂ wasÂ usedÂ toÂ inoculateÂ 2X60Â mlÂ ofÂ TBÂ mediaÂ containingÂ 50mg/mlÂ kanamycinÂ andÂ 50Â Ã¬g/mlÂ chloramphenicol,Â whichÂ wasÂ placedÂ inÂ aÂ 37Â°CÂ shakerÂ overnight.Â Â TheÂ nextÂ dayÂ thisÂ starterÂ cultureÂ wasÂ usedÂ toÂ inoculateÂ 12LÂ ofÂ TBÂ mediaÂ (10Â mlÂ starterÂ cultureÂ usedÂ perÂ 1L)Â containingÂ 50Â Ã¬g/mlÂ kanamycin.Â Â WhenÂ theÂ OD600Â reachedÂ approximatelyÂ 1.0Â theÂ

temperatureÂ wasÂ reducedÂ toÂ 18Â°CÂ andÂ afterÂ aÂ furtherÂ 30Â minutesÂ theÂ cellsÂ wereÂ inducedÂ byÂ theÂ additionÂ ofÂ 0.1Â mMÂ IPTG.Â

ExpressionÂ wasÂ continuedÂ overnight.

CellÂ harvest

CellsÂ wereÂ harvestedÂ byÂ centrifugationÂ atÂ 16,000Â RPMÂ afterÂ whichÂ theÂ supernatantÂ wasÂ pouredÂ outÂ andÂ theÂ cellÂ pelletÂ eitherÂ placedÂ inÂ aÂ -80Â°CÂ freezerÂ orÂ usedÂ directlyÂ forÂ purification.

Purification

Buffers Used:

Binding/LysisÂ Buffer:Â 50Â mMÂ HepesÂ (pHÂ 7.5),Â 500Â mMÂ NaCl,Â 5%Â Glycerol, Â 20Â mMÂ ImidazoleÂ pHÂ 7.5, 0.01mM TCEPÂ

WashÂ Buffer:Â 50Â mMÂ HepesÂ (pHÂ 7.5),Â 500Â mMÂ NaCl,Â 5%Â Glycerol,Â 40Â m MÂ ImidazoleÂ pHÂ 7.5, 0.01mM TCEPÂ

ElutionÂ Buffer:Â 50Â mMÂ HepesÂ (pHÂ 7.5),Â 500Â mMÂ NaCl,Â 5%Â Glycerol,Â 250 Â mMÂ ImidazoleÂ pHÂ 7.5, 0.01mM TCEP

Gel Filtration Buffer: 10Â mMÂ HepesÂ (pHÂ 7.5),Â 500Â mMÂ NaCl,Â 5%Â Glycerol, 0.01mM TCEP

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CellÂ Lysis

CellÂ pelletsÂ wereÂ dissolvedÂ inÂ approximatelyÂ 50mlÂ lysisÂ bufferÂ andÂ brokenÂ by Â passingÂ throughÂ theÂ homogeniserÂ (x6)Â atÂ aÂ

constantÂ pressureÂ ofÂ 15KPa.Â Â TheÂ cellÂ debrisÂ wasÂ pelletedÂ atÂ 16,000Â RPMÂ andÂ theÂ supernatantÂ usedÂ forÂ furtherÂ purification.

ColumnÂ 1

Ni-NTAÂ (5.0Â mlÂ volumeÂ inÂ aÂ gravity-flowÂ column).

TheÂ clarifiedÂ cellÂ extractÂ wasÂ incubatedÂ withÂ 5.0Â mlÂ pre-equilibriatedÂ 50%Â

Ni_NTAÂ beadÂ solutionÂ forÂ 1Â hourÂ atÂ 4Â°CÂ withÂ rotationÂ afterÂ whichÂ itÂ was Â passedÂ throughÂ aÂ glassÂ column.Â TheÂ columnÂ wasÂ thenÂ washedÂ withÂ 50mlÂ BindingÂ BufferÂ (2Â xÂ 25ml)Â andÂ 50Â mlÂ WashÂ BufferÂ (2Â x25Â ml).Â TheÂ prote inÂ wasÂ elutedÂ withÂ 50Â mlÂ ofÂ

ElutionÂ BufferÂ inÂ 5Â xÂ 5Â mlÂ fractions.Â

ColumnÂ 2

SuperdexÂ s200Â 16/60Â GelÂ Filtration.Â

Elution fraction 1 and 2 were then pooled and concentrated to 5 ml
(10Â kDaÂ mwcoÂ concentrator) and applied to the GF column (pre-equlibriated in GF buffer) at 1.0 ml/min. 1.0 ml fractions were collected.

EnzymaticÂ treatmentÂ andÂ purificationÂ

The N-terminal His6- tag was cleaved by incubating overnight with TEV (20Â°C). Cleaved protein was purified by batch binding on 1ml pre-equilibriated 50% Ni-NTA bead solution. The column was then washed with 2x1ml Gel Filtration buffer, 2x1ml Wash buffer.

Concentration

ToÂ setÂ upÂ platesÂ theÂ sampleÂ wasÂ concentratedÂ toÂ 15.39Â mg/mlÂ usingÂ Â aÂ 10 Â kDaÂ mwcoÂ concentrator.

MassÂ spectrometryÂ characterisation

ExpectedÂ Â mass: 17373.7 DaÂ

MeasuredÂ mass: 17361.1271Â Â Da

Crystallization

Crystals were grown by vapour diffusion in sitting drop at 4Â°C. A sitting drop consisting of 50 nl protein and 100nl well solution was equilibrated against well solution containing 20%(w/v) PEG 3350 -- 0.2M sodium malonate.

DataÂ collectionÂ

Resolution:Â Â 2.6Â Ã□Â Â

X-rayÂ source:Â DiamondÂ LightÂ SourceÂ beamlineÂ IO3