SCMH1

PDB:2P0K

Revision

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Entry Clone Accession:BC009752
Entry Clone Source:MGC AU46-E6
SGC Clone Accession:SCMH1_1:G11-APC047
Tag:N-terminal hexahistidine tag with integrated TEV protease cleavage site: mhhhhhhssgrenlyfq*g
Host:E Coli BL21(DE3) RIL CodonPlus

Construct

Prelude:
Sequence:

mhhhhhhssgrenlyfqgMLVCYSVLACEILWDLPCSIMGSPLGHFTWDKYLKETCSVPAPVHCFKQSYTPPSNEFKISMKLEAQDP
RNTTSTCIATVVGLTGARLRLRLDGSDNKNDFWRLVDSAEIQPIGNCEKNGGMLQPPLGFRLNASSWPMFLLKTLNGAEMAPIRIFH
KEPPSPSHNFFKMGMKLEAVDRKNPHFICPATIGEVRGSEVLVTFDGWRGAFDYWCRFDSRDIFPVGWCSLTGDNLQPPGTK

Vector:p28a-mhl

Growth

Medium:TB
Antibiotics:
Procedure:A glycerol stock was used to innoclate 20 mL LB media containing 50 µg/mL kanamycin. The culture was grown overnight at 37ºC with shaking. The next day this starter culture was used to innoculate 1L of TB medium which contained 50 microG/mL kanamycin. The culture was grown in LEX at 37ºC to OD600 ~1.1 and was induced with the addition of 0.5 mM IPTG. The temperature was reduced to 15ºC and the culture was incubated for a further 16 hours before harvesting the cells.

Purification

Procedure
Column 1: Affinity purification, open Ni-NTA column
Procedure: The supernatant was incubated with 6ml of 50% slurry Talon beads by rocking. After 2 hour incubation at 4ºC the protein was successfully bound to beads. The beads were washed twice with 50 ml of each WBI and WBII. The protein was eluted using 20ml EB and 2mM DTT was added to the eluate. Note that protein was precipitating in the EB, thus the buffer conditions

were changed for gel filtration by dialysis in the GF buffer using membrane with 3500 Da molecular weight cut off.

Column 2: Gel filtration, HiLoad 26/60 Superdex 200 Prep Grade
Procedure: The dialyzed protein was loaded onto the gel filtration column in GF buffer at 2.5 mL/min, fraction size 4ml. The fractions containing protein were identified on a SDS-PAGE gel.

Extraction

Procedure
Cells were harvested by centrifugation and pellets were stored in -80ºC. Prior to purification, the cell pellet was resuspended in lysis buffer. Cells were disrupted by sonication 10 minutes twice and samples were centrifuged for 60 min at 70000 g. The soluble fraction was subjected to further purification by affinity and size exclusion chromatography.
Concentration: The concentration of SCMH1 in GF buffer was determined to be 16 mg/mL by standard Bradford assay.
Ligand
MassSpec:
Crystallization: Vapour diffusion, sitting drop method was used. Crystals were obtained by mixing 0.5 microL of protein and 0.5 microL of well solution containing 2.0M ammonium phosphate and 0.1M Tris-HCl, pH 8.5 at 20ºC
NMR Spectroscopy:
Data Collection: Resolution: 1.75 Å, X-ray source: Synchrotron APS beamline 17-ID, single wavelength
Data Processing: