MGC45594

PDB:2C0C

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:MGC45594A-s001
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal His-tag with TEV protease cleavage site
Host:E.coli strain BL21DE3- R3

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfqsmMQKLVVTRLSPNFREAVTLSRDCPVPLPGDGDLLVRNRFVGVNASDINYSAGRYDPSVKPPFDI
GFEGIGEVVALGLSASARYTVGQAVAYMAPGSFAEYTVVPASIATPVPSVKPEYLTLLVSGTTAYISLKELGGLSEGKKVLVTAAAG
GTGQFAMQLSKKAKCHVIGTCSSDEKSAFLKSLGCDRPINYKTEPVGTVLKQEYPEGVDVVYESVGGAMFDLAVDALATKGRLIVIG
FISGYQTPTGLSPVKAGTLPAKLLKKSASVQGFFLNHYLSKYQAAMSHLLEMCVSGDLVCEVDLGDLSPEGRFTGLESIFRAVNYMY
MGKNTGKIVVELPH

Vector:pNIC28-BSA4

Growth

Medium:
Antibiotics:
Procedure:

Purification

Procedure
Column 1: Ni-NTA resin.

Procedure: The clear supernatant after centrifugation was passed through a Ni-NTA (2.5mL resin) column twice. The column was washed with 50 mL of wash buffer (500 mM NaCl, 5% Glycerol, 50 mM Tris-HCl pH 7.5, 30 mM Imidazole), and protein was eluted with 15 mL of elution buffer (500 mM NaCl, 5% Glycerol, 50 mM HEPES pH 7.5, 250mM Imidazole).

Column 2 : Hiload 16/60 Superdex 200 prep grade 120 mL, GE Healthcare.

Buffer : 10 mM HEPES, pH 7.5, 500 mM NaCl, 5 % glycerol, 0.5 mM TCEP.

Procedure: The eluted fractions from the Ni-affinity HisTrap columns were loaded on the gel filtration column in GF buffer at 1.0 mL/min. Eluted proteins were collected in 1 mL fractions

Concentration : 5 mg/mL using Vivaspin 10K concentrators

Extraction

Procedure

Concentration:
MassSpec:
Crystallization: Column 1: Ni-NTA resin.

Procedure: The clear supernatant after centrifugation was passed through a Ni-NTA (2.5mL resin) column twice. The column was washed with 50 mL of wash buffer (500 mM NaCl, 5% Glycerol, 50 mM Tris-HCl pH 7.5, 30 mM Imidazole), and protein was eluted with 15 mL of elution buffer (500 mM NaCl, 5% Glycerol, 50 mM HEPES pH 7.5, 250mM Imidazole).

Column 2 : Hiload 16/60 Superdex 200 prep grade 120 mL, GE Healthcare.

Buffer : 10 mM HEPES, pH 7.5, 500 mM NaCl, 5 % glycerol, 0.5 mM TCEP.

Procedure: The eluted fractions from the Ni-affinity HisTrap columns were loaded on the gel filtration column in GF buffer at 1.0 mL/min. Eluted proteins were collected in 1 mL fractions

Concentration : 5 mg/mL using Vivaspin 10K concentrators
NMR Spectroscopy:
Data Collection:
Data Processing: