FLJ20604A

PDB:2C9H

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:FLJ20604A-c010
Entry Clone Source:
SGC Clone Accession:
Tag:N-terminal Histag fusion to FLJ20604 with the following sequence:
mhhhhhhssgvdlgtenlyfqsm
Host:BL21(DE3)- R3

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfqsmRLHRRVVITGIGLVTPLGVGTHLVWDRLIGGESGIVSLVGEEYKSIPCSVAAYVPRGSDEGQFN
EQNFVSKSDIKSMSSPTIMAIGAAELAMKDSGWHPQSEADQVATGVAIGMGMIPLEVVSETALNFQTKGYNKVSPFFVPKILVNMAA
GQVSIRYKLKGPNHAVSTACTTGAHAVGDSFRFIAHGDADVMVAGGTDSCISPLSLAGFSRARALSTNSDPKLACRPFHPKRDGFVM
GEGAAVLVLEEYEHAVQRRARIYAEVLGYGLSGDAGHITAPDPEGEGALRCMAAALKDAGVQPEEISYINAHATSTPLGDAAENKAI
KHLFKDHAYALAVSSTKGATGHLLGAAGAVEAAFTTLACYYQKLPPTLNLDCSEPEFDLNYVPLKAQEWKTEKRFIGLTNSFGFGGT
NATLCIAGL

Vector:pNIC28-BSA4

Growth

Medium:
Antibiotics:
Procedure: The cells were grown at 30°C in 1 liter Overnight Expression Instant TB Medium (Novagen) containing 50 µg/ml kanamycin for 26 hours before the cells were harvested by centrifugation.

Purification

Procedure
Column 1 : Ni_NTA batch column (Qiagen), 5 ml of 50% slurry in 1.5 x 10 cm column.
Buffers: Washing buffer: 500 mM NaCl, 5% glycerol, 50 mM Tris-HCl pH 7.5, 30 mM imidazole, 2 mM TCEP. Elution buffer: 500 mM NaCl, 5% glycerol, 50 mM HEPES pH 7.5, 250 mM imidazole, 2mM TCEP.
Procedure: After the supernatant was loaded to the Ni-NTA column equilibrated with the

extraction buffer, the column was washed with 100ml washing buffer, and FLJ20604A was eluted in 15 ml elution buffer.

Column 2 : Hi Load 16/60 Superdex 200
Buffers : 300 mM NaCl, 10% glycerol, 10 mM HEPES pH 8.0, 2mM TCEP

Procedure: The eluted fraction was loaded to the Superdex column to change the buffer and achieve homogenous FLJ20604A for crystallization. The protein was concentrated using a 30000 MW cutoff Amicon Ultra concentration device.

Extraction

Procedure
Extraction buffer: 500 mM NaCl, 5% glycerol, 50 mM HEPES pH 7.5, 5 mM imidazole, 2 mM TCEP. Cell pellets from 1 liter were resuspened in 50 ml extraction buffer and then lysed by use of a French Press. The lysate was centrifuged at 18,000 RPM for 1 hour. The cleared lysate was loaded to the Ni-NTA column.
Concentration:
Ligand
MassSpec:
Crystallization: Column 1 : Ni_NTA batch column (Qiagen), 5 ml of 50% slurry in 1.5 x 10 cm column.
Buffers: Washing buffer: 500 mM NaCl, 5% glycerol, 50 mM Tris-HCl pH 7.5, 30 mM imidazole, 2 mM TCEP. Elution buffer: 500 mM NaCl, 5% glycerol, 50 mM HEPES pH 7.5, 250 mM imidazole, 2mM TCEP.
Procedure: After the supernatant was loaded to the Ni-NTA column equilibrated with the extraction buffer, the column was washed with 100ml washing buffer, and FLJ20604A was eluted in 15 ml elution buffer.

Column 2 : Hi Load 16/60 Superdex 200
Buffers : 300 mM NaCl, 10% glycerol, 10 mM HEPES pH 8.0, 2mM TCEP

Procedure: The eluted fraction was loaded to the Superdex column to change the buffer and achieve homogenous FLJ20604A for crystallization. The protein was concentrated using a 30000 MW cutoff Amicon Ultra concentration device.
NMR Spectroscopy:
Data Collection:
Data Processing: