CGI63

PDB:1ZSY

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:CGI 63A-s001 (gi|7705777)
Entry Clone Source:MGC
SGC Clone Accession:
Tag:
Host:

Construct

Prelude:
Sequence:

mhhhhhhssggvdlgtenlyfqSMPARVRALVYGHHGDPAKVVELKNLELAAVRGSDVRVKMLAAPINPSDINMIQGNYGLLPELPA
VGGNEGVAQVVAVGSNVTGLKPGDWVIPANAGLGTWRTEAVFSEEALIQVPSDIPLQSAATLGVNPCTAYRMLMDFEQLQPGDSVIQ
NASNSGVGQAVIQIAAALGLRTINVVRDRPDIQKLSDRLKSLGAEHVITEEELRRPEMK

Vector:pNIC-Bsa4

Growth

Medium:
Antibiotics:
Procedure: Medium TB + 50 µg/mL Kanamycin

1 L TB in 2.5-L baffled flasks was inoculated with 10 mL overnight culture. The culture was grown at 37°C to OD=2.3, and transferred to 25°C. 1 mM IPTG was then added, and incubation continued for 4 hours. The cells were then collected by centrifugation and frozen at -80°C.

Purification

Procedure
Column 1 : Ni-affinity, HisTrap, 1 mL (GE/Amersham)

Buffers: Lysis buffer: 50 mM Tris-HCl, pH 7.5, 500 mM NaCl, 10 mM imidazole, 0.5 mM TCEP; Wash buffer: 50 mM Tris-HCl, pH 7.5, 500 mM NaCl, 50 mM imidazole, 0.5 mM TCEP; Elution buffer: 50 mM Tris-HCl, pH 7.5, 500 mM NaCl, 250 mM imidazole, 0.5 mM TCEP. Procedure: The cell extract was loaded on the column at 0.8 mL/minute on an AKTA-express system (GE/Amersham). The column was then washed with 10 volumes of Lysis buffer, 10

volumes of wash buffer, and then eluted with elution buffer at 0.8 mL/min. The eluted peak of A280 was automatically collected.

Column 2 : Gel filtration, Hiload 16/60 Superdex 200 prep grade 120 mL, Code no. 17-1069-01 Amersham Biosciences

Buffers: GF 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 : Vivaspin 6 mL (10K MWC) to 20mg/mL.

Extraction

Procedure
Lysis buffer: 50 mM Tris-HCl, pH 7.5, 500 mM NaCl, 10 mM imidazole, 0.5 mM TCEP, Complete® protease inhibitors (1 tablet/50 mL). Frozen cell pellets were thawed on ice over night and resuspended in a total volume of 40 mL lysis buffer, the cells were lysed by high pressure (20 psi) followed by sonication. Nucleic acids and cell debris were removed by adding 0.15% PEI from a 5% (w/v) stock, stirring for 15 minutes, then centrifugation for 20 minutes at 40,000 x g. The supernatant was then further clarified by filtration (0.45 µm).
Concentration:
Ligand
MassSpec:
Crystallization: Column 1 : Ni-affinity, HisTrap, 1 mL (GE/Amersham)

Buffers: Lysis buffer: 50 mM Tris-HCl, pH 7.5, 500 mM NaCl, 10 mM imidazole, 0.5 mM TCEP; Wash buffer: 50 mM Tris-HCl, pH 7.5, 500 mM NaCl, 50 mM imidazole, 0.5 mM TCEP; Elution buffer: 50 mM Tris-HCl, pH 7.5, 500 mM NaCl, 250 mM imidazole, 0.5 mM TCEP. Procedure: The cell extract was loaded on the column at 0.8 mL/minute on an AKTA-express system (GE/Amersham). The column was then washed with 10 volumes of Lysis buffer, 10 volumes of wash buffer, and then eluted with elution buffer at 0.8 mL/min. The eluted peak of A280 was automatically collected.

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NMR Spectroscopy:
Data Collection:
Data Processing: