RGS17

PDB:1ZV4

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:RGS 17A-s001
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal hexahistidine tag with integrated TEV protease cleavage site: mhhhhhssgdlgtenlyfq*sm
Host:BL-21(DE3)R3 (phage resistant)

Construct

Prelude:
Sequence:

mhhhhhssgdlgtenlyfqsmNPTAEEVLSWSQNFDKMMKAPAGRNLFREFLRTEYSEENLLFWLACEDLKKEQNKKVIEEKARMIY
EDYISILSPKEVSLDSRVREVINRNLLDPNPHMYEDAQLQIYTLMHRDSFPRFLNSQIYKSFVESTAGS

Vector:p21a-LIC

Growth

Medium:
Antibiotics:
Procedure:2 µL of the construct was added and mixed to 100 µL of BL21(DE3)-Phage Resistant competent cells in a sterile 96-well microtitre plate on ice. The plate was left on ice for a further 30 minutes. The heat-shock procedure was done by transferring the plate to a 42°C water bath for 45 seconds and then returning it to sit on ice for 2 minutes. 100 µL of LB medium (pre-warmed to 42°C) was added to the well and the plate incubated at 37°C for 30 minutes. The entire 202 µL culture was plated out onto LB-Amp agar in a 5.5 cm Petri dish. The plates were incubated at 37°C overnight. Glycerol stock: Three colonies were picked into 1.5 mL of LB media, with 100µg/mL Ampicillin, in a 96-well plate and placed in a shaker overnight at 37°C. A 80 µL sample was removed from the well into a new 96-well plate. 40 µL 75 % Glycerol (sterile) was added before freezing in a -80°C freezer. 1L growth: The RGS 17A-c006 glycerol stock was used to innoclate 20 mL LB media with 100 µg /mL Ampicillin which was placed in a 37°C shaker overnight. The next day this starter culture was used to innoculate 1 litre of TB medium which contained 100 µg/mL Ampicillin. Protein induction was carried out with the addition of 0.5 mM IPTG after the cells reached an OD600 of 0.35 and the incubation temperature decreased to 25°C. After 4 hours the cells were harvested by centrifugation. The cell pellet was resuspended in approximately 40 mL of Lysis Buffer before being frozen in the -80°C freezer. Lysis Buffer: 20 mM Tris pH8.0, 200 mM NaCl, 5 % Glycerol, 10 mM Imidazole.

Purification

Procedure
Column 1 : Low pressure chromatography using Bio-Rad Econo column (2.5 cm x 13 cm).

Buffers: Wash Buffer I (WBI): 50 mM Hepes pH 8.0, 500 mM NaCl, 5 % glycerol, 5 mM imidazole pH 8.0, 0 .5 mM TCEP; Wash Buffer II (WBII): 50 mM Tris pH 8.0, 500 mM NaCl, 5 % glycerol, 25 mM imidazole pH 8.0, 0.5 mM TCEP; Elute Buffer (EB): 50 mM Hepes pH 8.0, 500 mM NaCl, 5 % glycerol, 250 mM imidazole pH 8.0, 0.5 mM TCEP.

Procedure: Total volume of Ni-NTA added to BioRad drip column: 4 mLs (50 %). Resin washed with 12.5 mL of WBI. The supernatant was applied to a column using 5 mL pipette and allowed to pass over the resin. The flow through was collected in a 50 mL falcon tube and applied once more to the column. Two wash steps followed. Wash with 12.5 mL of WBI. Wash with 12.5 mL column vols of WBII. Elute with 14 mLs of EB into 7x2 mL fractions.

Column 2 : Size exclusion using a S75 16/60 column

Extraction

Procedure
1 tablet protein inhibitor in 10 mL Lysis Buffer was added to the 1L growth pellet. Total vol: 45 mLs (estimate). Cell breakage: 5 passes through the Emulsiflex C5 high pressure homogeniser. Total vol: 50 mLs (estimate). Centrifuge for 30 mins at 16000 rpm and 4°C to remove cell debris. Discard pellet.
Concentration:
Ligand
MassSpec:
Crystallization: Hanging drops were setup up in a Linbro plate by mixing 1 volume of protein at 20.3 mg/mL in 10 mM Borax pH 9.0, 500 mM NaCl with 1 volume of reservoir solution: 1M Na Succinate pH 7.0, 1 % mPEG2K, 0.1M Hepes pH 7.0 . The drop was seeded directly after setup with a cat whisker after touching the surface of a small crystal of RGS 17 previously obtained under the same conditions.
NMR Spectroscopy:
Data Collection:
Data Processing: