RGS2

PDB:2AF0

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:RGS2A-s001
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal hexahistidine tag
Host:BL-21(DE3)R3 phage resistant

Construct

Prelude: The valine marked in red in the sequence has been modelled as Alanine in the deposited PDB strutcure.
Sequence:

MHHHHHHSSGVDLGTENLYFQSMKPSPEEAQLWSEAFDELLASKYGLAAFRAFLKSEFCEENIEFWLACEDFKKTKSPQKLSSKARK
IYTDFIEKEAPKEINIDFQTKTLIAQNIQEATSGCFTTAQKRVYSLMENNSYPRFLESEFYQDLCKKPQITTEPHAT

Vector:pNIC28-Bsa4

Growth

Medium:
Antibiotics:
Procedure:10 microL of RGS 2A-c230 DNA was pipetted into a well of a 96-well plate. Added 50 µl competent BL-21(DE3)R3 cells before incubating on ice for 30 minutes. Next the cells were heat shocked for 42 seconds at 42°C in a water bath before returning onto ice for a further 2 minutes. 100 µl of SOC medium was added and the plate incubated for 1 hour at 37°C. A total of 135 µl of the cell suspension was plated onto an LB agar plate that contained 50 µg/ml kanamycin.

Glycerol stocks: Colonies from the transformation were used to inoculate 1 ml TB containing 50 µg/ ml kanamycin. The cells were then grown at 700 RPM , 37° C in a GlasCol shaker for approximately 12 hrs. Next, 250 µ l of 60% glycerol was added to the culture, mixed and 50 µl pipetted into a well of a 96-well plate before storing at -80°C.
Using the glycerol stock, 10 ml of TB + 50 µg/ml kanamycin was used inoculated and grown overnight. This was used as a starter culture for a 1 litre culture of TB + 50 µg/ml kanamycin. The culture was grown at 37°C, transferred to 25°C when the OD600 reached a value of 2. The culture was grown at this temperature until OD600 reached approximately 3 when protein production was induced with the addition of 1 mM IPTG. The cells were then grown overnight at 25°C. The next day the cells were collected by centrifugation and frozen at -80°C.

Purification

Procedure
Column 1 : Ni-affinity, HisTrap, 1 ml (GE/Amersham)
Procedure: The cell extract was loaded on the column at 0.8 ml/min on an AKTA-express system (GE/Amersham). The column was then washed with 10 column volumes of Lysis buffer, 10 column 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
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 : Using a 2 ml Vivaspin 3 K cutoff concentrator the RGS 2A-p001 was concentrated to 30 mg/ml. Concentration was determined from the absorbance at 280 nm.

Extraction

Procedure
Frozen cell pellets were thawed on ice over night and resuspended in a total volume of 50 ml lysis buffer. The cells were disrupted by four passes at 20,000 psi through a high pressure homogeniser followed by sonication. Nucleic acids and cell debris were removed by adding 0.15 % PEI , stirring for 15 minutes, then centrifugation for 20 minutes at 40,000xg. The supernatant was then further clarified by filtration (0.45 µm).
Concentration:
Ligand
MassSpec: Calculated mass of the full length construct is 19040 Da and the determined mass was 19039 Daltons .
Crystallization: Crystals grew from a 2:1 ratio mix of RGS 2A-to-reservoir (2.0 M ammonium sulphate, 0.2M NaCl, 0.1 M cacodylate pH 6.5).
NMR Spectroscopy:
Data Collection: Resolution: 2.3Å, X-ray source: Synchrotron SLS -X10, single wavelength.
Data Processing: