RGS16

PDB:2BT2

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:RGS 16-s001
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal: His-tag with integrated thrombin protease site:
MHHHHHHSSGVDLGTENLYFQ*SH
Host:BL-21(DE3)

Construct

Prelude:
Sequence:

Vector:pLIC-SGC1

Growth

Medium:
Antibiotics:
Procedure: The construct was transformed into E. coli BL21(DE3) cells and grown as a 1mL Terrific Broth + 100 µg/mL ampicillin culture overnight at 37°C with shaking at 700 rpm. This was used to prepare a glycerol stock by adding glycerol to a final concentration of 15 % before storage at -80°C. Using the frozen glycerol stock a 1 litre Terrific Broth + 100 µg/mL ampicillin culture was inoculated. The cultures were induced at approximately 2.3 OD600 with 1.0 mM IPTG at 25 degrees for 4 hours (shaking at 200 rpm) and harvested on the same day. The pellets were frozen at -80°C.

Purification

Procedure

Extraction

Procedure
Cell Lysis - Lysis Buffer: 50 mM Tris/ HCl pH 7.5, 500 mM NaCl, 10 mM imidazole, 0.5 mM

TCEP. 1 tablet protein inhibitor in 25 mL Lysis Buffer was added to the 1L growth pellet. Total vol: 40 mL (estimate). Cell breakage: 5 passes through the Emulsiflex C5 high pressure homogeniser, brief sonication on ice (10s bursts). Precipitation of DNA: Addition of PEI (final conc. 0.15%), followed by incubation for 10 min. at 4°C. Total vol: 50 mL (estimate). Removal of cell debris: After centrifugation for 30 mins at 17000 rpm and 4°C the pellet was discarded, and the supernatant filtered (0.45 μm).

Concentration:

Ligand

MassSpec:

Crystallization: Crystals grew from a 1:2 ratio mix of RGS16A-to-reservoir (26 % PEG3350, 0.1 M ammonium acetate, 0.1 M Bis-Tris pH 5.5).

NMR Spectroscopy:

Data Collection:

Data Processing: