RGS8

PDB:2IHD

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:RGS8A-s001
Entry Clone Source:IMAGE
SGC Clone Accession:
Tag:mhhhhhhssgvdlgtenlyfq*s(m) TEV-cleavable (*) N-terminal his6 tag.
Host:BL21 (DE3)R3 (Phage resistant strain)

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfqsmLKRLST EEATRWADSFDVLLSHKYGVAAFRAFLKT EFSEENLEFWLACEEFKKTRSTAKLVS
KA HRIFEEFVDVQAPREVNIDFQTREATRKN LQEPSLTCFDQAQGKVHSLMEKDSYPRFL RSKMYLDLLS

Vector: Vector: pNIC28-Bsa4

Growth

Medium:
Antibiotics:
Procedure: Using a glycerol stock of the transformed cells, a starter culture (50 ml of TB plus 50 µg/ml kanamycin) was grown overnight at 37°C (200 rpm). 10 mls of this culture was used to inoculate 3 x 1 litres of Terrific Broth plus 50 µg/ml kanamycin. The cells were further grown at 37°C (200 rpm) until the culture reached an OD600 of between 2-3. At this stage the temperature of the incubator was dropped to 18°C for 1/2 hour before induction of protein synthesis by the addition of 0.2 mM IPTG. Total induction time was ~16 hour after which the cells were pelleted by centrifugation at 6000 rpm for 20 minutes at 4°C. The pellets were then resuspended in stored in a -80°C freezer until further use.

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 Binding Buffer, 10 column volumes of Wash Buffer and the RGS8 domain eluted with Elution Buffer at 0.8 ml/min. The eluted peak of A280 was automatically collected.

Column 2 : Gel filtration, Hiload 16/60, S75 16/60 - 120 ml
The eluted fractions from the Ni-affinity Histrap columns were loaded on the gel filtration column in Gel Filtration Buffer at 1.0 ml/min. Eluted proteins were collected in 1 ml fractions.

Concentration : The eluted fractions that contained the RGS8 domain as judged by SDS-PAGE were pooled and concentrated to 10 mg/ml using a Millipore 10 kD cutoff spin concentrator. The concentrated protein was distributed into 7 x 50 m l aliquots before freezing in a -80°C freezer.

Extraction

Procedure
The 3 litre pellets were resuspended in ~100 ml lysis buffer. The cells were lysed by passing through a high pressure homogeniser (EmulsiFlex C5), 4 cycles, bringing the final volume to ~150 ml .
PEI (stock 5%) was added to the homogenate to a final concentration of 0.15%. The cell debris, nuclei and DNA were spun down at 16,500 rpm for 60 min (rotor JA 17). The supernatant was collected and filtered through a 0.22 µm filter, using a pump. The last drops that didn't go through were filtered manually through two filters 1.2 plus 0.22µm on a syringe.
Concentration:
Ligand
MassSpec: Expected MWt: 18202.4; Measured MWt: 18203.
Crystallization: Crystals grew from a 1:1 ratio mix of MPDZA-p001-to-reservoir (0.2 M NaI; 20 % PEG 3350 ; 10 % ethylene glycol)
NMR Spectroscopy:
Data Collection: Resolution: 1.7 Å; X-ray source: Synchrotron SLS-X10, single wavelength.
Data Processing: