# RGS1 + G alpha I: Human regulator of G-protein signalling 1 in complex with the activated state of G alpha I

**PDB:**2GTP

## Revision

**Revision Type:**created
**Revised by:**created
**Revision Date:**created
**Entry Clone Accession:**
**Entry Clone Source:**Prof D Siderovski
**SGC Clone Accession:**
**Tag:**
**Host:**

## Construct

**Prelude:**
**Sequence:**

MREVKLLLLGAGESGKSTIVKQMKIIHEAGYSEEECKQYKAVVYSNTIQSIIAIIRAMGRLKIDFGDSARADDARQLFVLAGAAEEG
FMTAELAGVIKRLWKDSGVQACFNRSREYQLNDSAAYYLNDLDRIAQPNYIPTQQDVLRTRVKTTGIVETHFTFKDLHFKMFDVGGQ
RSERKKWIHCFEGVTAIIFCVALSDYDLVLAEDEEMNRMHESMKLFDSICNNKWFTDTSIILFLNKKDLFEEKIKKSPLTICYPEYA
GSNTYEEAAAYIQCQFEDLNKRKDTKEIYTHFTCATDTKNVQFVFDAVTDVIIKNNLKDCGLF

**Vector:**pProEXHT

## Growth

**Medium:**
**Antibiotics:**
**Procedure:**Transformation: 2 microL of the construct was added and mixed to 90 microLof phage-resistant BL21 (DE3) with the Rosetta plasmid and left on ice for 20 minutes.The heat-shock procedure was done by transferring the plate to a 42degC water bathfor 45 seconds and then returning it to ice for a further 2 minutes.100 microL of LB medium (pre-warmed to 42degC) was added to the well and the plate incubated at 37degC for 40 minutes.

Large scale expression:The RGS 1A-c004 fresh transformants were used to innoculate 20 mL LB media with 100 microG/mL Ampicillinwhich was placed in a 37degC shaker overnight.The next day this starter culture was used to innoculate 4 x 1 litre of TB medium which contained 100 microG/mLAmpicillin.Protein induction was carried out with the addition of 0.5 mM IPTG after the cellsreached an OD600 of 0.7 and the incubation temperature decreased to 25degC.After 4 hours the cells were harvested by centrifugation. The cell pellet was frozen in the -80degC freezer.

## Purification

**Buffers**
Wash Buffer I (WBI): 50 mM Hepes pH 8.0, 300 mM NaCl, 5 % glycerol, 10 mM imidazoleWash Buffer II: 50 mM Tris pH 8.0, 300 mM NaCl, 5 % glycerol , 30 mM imidazoleElute Buffer: 50 mM Hepes pH 8.0, 300 mM NaCl, 5 % glycerol, 250 mM imidazole
**Procedure**
Total volume of Ni-NTA added to BioRad drip column: 4 ml (50 %). Resin washed with 12.5 mL of WBI.The supernatent 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 ml of EB into 7x2 ml fractions.

The fractions from gel filtration that contained G protein a1 were pooled and concentratedbefore loading on to S200 16/60 gel filtration column.The fractions containing protein were identified on a coomasie blue stained gel.

Enzymatic treatment : His-Tag removal. The eluate was treated with Tev protease. The sample was rebound to Ni sepharose to remove Tev and other contaminants. The His tag cleaved protein was concentrated to 300 ÂµM.

Common steps:Purification of G protein a 1 and RGS 1A complex:Equimolar concentrations of G protein and RGS 1A was mixed and incubated at 4 degrees for 15 minutes.The sample was passed through S200 gelfiltration column which was prequilibratedwith 20 mM Hepes pH 7.5, 100 mM NaCl, 5 % glycerol, 2 mM DTT, 30 m M AlCl3, 100 mM GDP and 20 mM NaF.The proteins eluted as a complex was analysed using PAGE and the fractions were pooled and concentrated to 24 mg/mLand used for crystallisation set up.

## Extraction

**Buffers**
Lysis Buffer: 50 mM Hepes pH 7.5, 300 mM NaCl, 5 % Glycerol, 10 Mm imidazole
**Procedure**
1 tablet protein inhibitor in 10 mL Lysis Buffer was added to the 1L growth pellet.Total vol: 45 mls (estimate).Cell breakage: 3 passes through the Emulsiflex C5 high pressure homogeniser.Centrifuge for 45 mins at 16000 rpm and 4degC to remove cell debris.
**Concentration:**
**Ligand**
**MassSpec:**The expected mass of N terminally 30 aminoacids truncated GNAI1A-C001 without histidine tag is 37113.The experimentally determined mass was 37111.
**Crystallization:**Crystals grew from a 1:1 ratio mix of G protein a1 , RGS 1A and precipitant containing 0.2M NaNO 3 ; 0.1M BT Prop pH 6.5, 20 % PEG 3350; 10 % EtGly.
**NMR Spectroscopy:**
**Data Collection:**Resolution: 2.55Å; X-ray source: Synchrotron SLS-X10, single wavelength.
**Data Processing:**