# RACGAP1

**PDB:**2OVJ

## Revision

**Revision Type:**created
**Revised by:**created
**Revision Date:**created
**Entry Clone Accession:**RACGAP1A-s001
**Entry Clone Source:**MGC
**SGC Clone Accession:**
**Tag:**N-terminal, TEV cleavable hexahistidine tag
**Host:**BL-21 (DE3)R3 Rosetta strain

## Construct

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

smEGMLADFVSQTSPMIPSIVVHCVNEIE QRGLTETGLYRISGCDRTVKELKEKFLRV KTVPLLSKVDDIHAICSLLKDFLRNLK EP LLTFRLNRAFMEAAEITDEDNSIAAMYQA VGELPQANRDTLAFLMIHLQRVAQSPHTK MDVANLAKVFGPTIVAHAVPNPDP VTMLQ DIKRQPKVVERLLSLPLEYWSQFMMVE

**Vector:**

## Growth

**Medium:**
**Antibiotics:**
**Procedure:**The RACGAP1A-c011 glycerol stock was used to innoclate 20 ml LB media with 50 microG/ml kanamycin and 34 microG/ml chloramphenicol which was placed in a 37degC shaker overnight. The next day this starter culture was used to innoculate 1 litre of TB medium which contained 50 µg/ml kanamycin. When OD600 reached ~1.1 the temperature was shifted down from 37degC to 22degC for 1 hour before induction with the addition of 0.5 mM IPTG. Protein expression was allowed to carry on for a further 16 hours before harvest.

## Purification

**Procedure**
Column 1: IMAC Ni-NTA column purification
Procedure: The Supernatant containing the protein was loaded on to manually packed Ni sepharose beads column and washed with 30 ml of wash buffer and eluted in 6 fractions of 2 ml each using the high imidazole elute buffer. The eluate was pooled and concentrated using 10 KDa cutoff millipore filters and loaded on to Gelfiltration column

Column 2 : Gel filtration, Hiload 16/60, S75 16/60 - 120 ml
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. The fractions containing protein were identified on a coomasie blue stained gel.

Enzymatic treatment : Add 100 µl of the home produced TEV protease to fractions containing protein and left at 4°C overnight. The protein was rebound to Nickel beads for removing the His tag and the flow through containing RACGAP1A

Concentration : The concentration of RACGAP1A was determined to be 25 mg/ml

## Extraction

**Procedure**
Total vol of cell: 60 mls (estimate). Cell breakage: After thawing, the resuspended cells were lysed by passing through Emulsiflex C5 high pressure homogeniser. 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 16000 rpm for 45 min. The supernatant was collected. Discard pellet.
**Concentration:**
**Ligand**
**MassSpec:**The Mass of the purified protein was measured to be 22719.4 which matched with the expected mass of 22720.3
**Crystallization:**Crystals grew from 2:1 ratio mix of protein and precipitant containing 25 % PEG3350, 0.1M Bis Tris 5.5.
**NMR Spectroscopy:**
**Data Collection:**Resolution: 1.7 Å, X-ray source: Synchrotron SLS-X10, single wavelength
**Data Processing:**