

# REM1: Human RAS-like GTP-binding protein REM1 in complex with GDP

**PDB:**2NZJ

## Revision

**Revision Type:**created

**Revised by:**created

**Revision Date:**created

**Entry Clone Accession:**REM1A-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:**

**Vector:**pNIC28-Bsa4

## Growth

**Medium:**

**Antibiotics:**

**Procedure:**The RRADA-c008 glycerol stock was used to innoculate 20 ml LB media with 50 µg/ml kanamycin and 34 µg/ml chloramphenicol which was placed in a 37°C 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 the temperature was shifted down from 37°C to 18°C for 1 hour before induction with the addition of 0.6 mM IPTG. Protein expression was allowed to carry on for a further 16 hours before harvest.

**Cell harvest:** Cells were spun at 4000 rpm for 10 mins at 4°C. The pellets resuspended in 25mls Resuspension Buffer. The resuspended cell pellets were placed in the -80°C freezer

## Purification

**Procedure**

## Extraction

## **Procedure**

### **Concentration:**

#### **Ligand**

**MassSpec:** The Mass of the purified protein was measured to be 19470.5 which matched with the expected mass of 19469.7.

**Crystallization:** Crystals grew from 1:1 ratio mix of precipitant containing 0.1M HEPES 7.7, 34% PEG3350, 0.26M NaCl and protein.

#### **NMR Spectroscopy:**

**Data Collection:** Resolution: 2.5 Å, X-ray source: Synchrotron SLS -X10, single wavelength.

#### **Data Processing:**