

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 inoculate 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 inoculate 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: