RHOD: Human Ras homolog gene family, member D

PDB:2J1L

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:BC001338
Entry Clone Source:MGC
SGC Clone Accession:
Tag: Tag sequence: mhhhhhhssgvdlgtenlyfqs*(m), TEV-cleavable (*), N-terminal his6 tag.
Host: Rosetta-R3

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfqsmAGEEAP PGVRSVKVVLVGDGGCGKTSLLMVFADGA FPESYTPTVFERYMVNLQVKGKPVHLH IW DTAGQDDYDRLRPLFYPDASVLLLCFDVT SPNSFDNIFNRWYPEVNHFCKKVPIIVVG CKTDLRKDKSLVNKLRRNGLEPVT YHRGQ EMA

Vector:pNIC28-Bsa4.

Growth

Medium:
Antibiotics:
Procedure: Medium: TB + 50 µg/ml Kanamycin + 34 ug/ml chloramp .
2 x 1 liter TB in 2.5-L baffled flasks were inoculated with 2 x 10 ml overnight culture and grown at 37°C. The protein expression was induced with 1 mM IPTG at OD600 = 3.3 at 18°C over night. The cells were collected by centrifugation and frozen at -80°C.

Purification

Procedure
Column 1 : Ni-affinity, HisTrap, 1 ml (GE/Amersham Biosciences)
The cell extract was loaded on the column at 0.8 ml/minute on an AKTA-express system (GE/Amersham). The column was then washed with 10 volumes of lysis buffer, 10 volumes of wash buffer, and then eluted with elution buffer at 0.8 ml/min. The eluted peak of A280nm was automatically collected.

Column 2 : Hiload 16/60 Superdex 75 prep grade 120 ml (GE/Amersham Biosciences)
The eluted fractions from the Ni-affinity Histrap columns were loaded on the gel filtration

column in GF buffer at 0.80 ml/min. Eluted proteins were collected in 2 ml fractions.

Concentration: A five fold molar excess of GDP , 5 mM DTT, 20 m M MgCl 2 and 10 mM imidazole was added to the diluted protein prior to concentration in Amicon (5 K) to 20 mg/ml. The protein concentration was determined spectrophotometrically using the predicted molar extinction coefficient 28420 (M-1 cm-1).

Extraction

Procedure
Frozen cell pellets from 2 liter were thawed at 37 o C and resuspended in a total volume of 100 ml lysis buffer. The cells were disrupted by high pressure (20 kpsi) and nucleic acids and cell debris removed by adding 0.15 % PEI , followed by centrifugation for 30 minutes at 40000 x g. The supernatant was further clarified by filtration (0.20 m m).
Concentration:
Ligand
MassSpec: The expected mass for RHODAp003 was 23981 Da. Two peaks of 24012.0 Da (+31 Da) and 23832 Da (-149 Da) were detected .
Crystallization: Crystals were grown by vapor diffusion at 20°C. A sitting drop consisting of 200 nl protein (20.0 mg/ml) and 200 nl well solution was equilibrated against well solution containing 0.4 M NH4Cl, 15 % ethylene-glycol, 19 % PEG6000 and 1 M MES pH 6. 15% ethylene-glycol was used as cryoprotectant for low temperature mounting.
NMR Spectroscopy:
Data Collection: Resolution: 2.5 Å; X-ray source: Synchrotron SLS -X10, single wavelength.
Data Processing: