Rab domain of predicted protein LOC201475 with bound GDP

PDB:2IL1

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:
Entry Clone Source: Openbiosystems
SGC Clone Accession:
Tag:N-terminal hexahistidine tag
Host:E.coli. BL21 (DE3) codon(+) RIL

Construct

Prelude:
Sequence:

MGSSHHHHHHSSGLVPRGSPRPADFKLQVIIIGSRGVGKTSLMERFTDDTFCEACKSTVGVDFKIKTVELRGKKIRLQIWDTAGQER
FNSITSAYYRSAKGIILVYDITKKETFDDLPKWMKMIDKYASEDAELLLVGNKLDCETDREITRQQGEKFAQQITGMRFCEASAKDN
FNVDEIFLKLVDDILKKM

Vector:p28a-thrombin-lic

Growth

Medium:
Antibiotics:
Procedure: We prepared the seeds by inoculating glycerol stock of E. coli cells BL21-CodonPlus (DE-3)-RIL into 100 mL of Luria-Bertani medium. After overnight growth, all of the seeds were inoculated into 1.8 L of Terrific Broth medium in the presence of 50 µg/mL of kanamycin and 50 µg/mL chloramphenicol at 37 ºC and grown to an OD600 between 3-5. Cells were then induced by IPTG at the final concentration of 1.0 mM and grown overnight at 18 ºC in the SGC LEX bubbling system.

Purification

Procedure

Extraction

Procedure
Cultures were centrifuged and the cell pellets were harvested and stored at -80 ºC before use. Cells were thawed and suspended in 100 mL binding buffer (10 mM Tris pH 7.5, 0.5 M NaCl, 5 mM imidazole), 1 mM phenylmethyl sulfonyl fluoride (PMSF), 0.5% (v/v) protease inhibitor cocktail (Sigma), 1 mM Benzamidine, 1600 units Benzonase (Sigma), and lysed with sonication. The lysate was centrifuged at 16000 rpm for 45 min and the supernatant was used for subsequent steps of purification. All the extraction steps were carried out at 4 ºC.
Concentration:
Ligand
MassSpec:
Crystallization: Crystals were obtained using the vapor diffusion method and a protein concentration of 60 mg/ml containing 5 molar fold of GDP and MgCl2. 0.5 µl of the concentrated protein mixed with 0.5 µl of a well solution containing 22% PEG4000, 0.2M calcium acetate, 0.1M sodium cacodylate, pH 5.0. Crystals appeared after overnight incubation at 18°C.
NMR Spectroscopy:
Data Collection: Crystals were cryo-protected using 50% mineral oil and 50% parotone, and flash frozen in liquid nitrogen. Diffraction data were collected on our Rigaku FR-E Superbright diffractometer to 2.1 Å.
Data Processing: