RABGAP1L

PDB:3HZJ

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:
Entry Clone Source: Codon Devices Synthesized: SGC cDNA library: 27-B3
SGC Clone Accession:HPC094-F01
Tag:N-terminal tag: MHHHHHHSSGRENLYFQ*G
Host:BL21-V2R-pRARE2

Construct

Prelude:HHL:E507-L815
Tag was removed.
Sequence:

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gEKILYSWGELLGKWHSNLGARPKGLSTLVKSGVPEALRAEVWQLLAGCHDNQAMLDRYRILITKDSAQESVITRDIHRTFPAHDYF
KDTGGDGQESLYKICKAYSVYDEDIGYCQGQSFLAAVLLLHMPEEQAFCVLVKIMYDYGLRDLYRNNFEDLHCKFYQLERLMQEQLP
DLHSHFSDLNLEAHMYASQWFLTLFTAKFPLCMVFHIIDLLLCEGLNIIFHVALALLKTSKEDLLQADFEGALKFFRVQLPKRYRAE
ENARRLMEQACNIKVPTKKLKKYEKEYQTMRESQLQQEDPMDRYKFVYL
```

Vector:pET28-mhl (GI:134105571)

Growth

Medium:
Antibiotics:
Procedure:LEX Bubbling. The target protein was expressed in E. coli by inoculating 100 mL of overnight culture grown in Luria-Bertani medium into a 1.8 L of Terrific Broth medium in the presence of 50 µg/mL kanamycin and 25 µg/mL chloramphenicol at 37 degC. When OD600 reached ~3.0, the temperature of the medium was lowered to 15 degC and the culutre was induced with 0.5 mM IPTG. The cells were allowed to grow overnight before harvested and flash frozen in liquid nitrogen and stored at -80 degC. For selenomethionine (SeMet) labeling, prepackaged M9 SeMET growth media kit (Medicilon) was used following manufacturer instructions.

Purification

Procedure
The lysate was centrigued at 15,000 rpm for 45 minutes and the supernatants were mixed with 5 mL 50% flurry of Ni-NTA beads and incubated at 4 degC on rotary shaker for one hour. The

mixture was then centrifuged at 2300 rpm for 5 min and the supernant discarded. The beads were then washed with washing buffer containing 30 mM and 75 mM Imidazole, and finally the elution buffer. The flow-trough was collected and further purifed by a Superdex-75 gel filtraton column pre-equilibrated with gel filtration buffer. Fractions were collected and digested with TEV protease. TEV protease was removed from the treated protein sample by adding 100 uL 50% flurry of Ni-NTA beads and the sample was purified with supderdex-75 gel filtration again. Fractions containing the protein were collected and concentrated with Amicon Ultra-15 centrifugal filter. The purify of the preparation is tested by SDS-PAGE to be around 95%.

Extraction

Procedure
Frozen cells from 1.8L TB culture were thawed and resuspended in 150 mL extraction buffer with freshly added 0.5% CHAPS, and supplemented with protease inhibitor cocktail (SIGMA Catalog # P8849), and 3 µL benzonase (Sigma Catalog # E1014, 250U/µL), and lysed using microfluidizer at 15,000 PSI.
Concentration:32.9 mg/mL for Native, 21.8 mg/mL for SeMet
Ligand
MassSpec: Mass of SeMet 38857.3 and Native expected 38428.2, measured 38434.8 and 38505.3 (delta of 70.5)

Mass of SeMet cut, measured 36713.9
Native expected 36291.88, measured 36293.4
Crystallization: Crystallization was setup using sitting drops with Red Wings and SGC-I screens initially. SeMet crystal used for structure determination was grown in 15% PEG3350, 0.2M KCl, pH 5.8 with 1:100 Dispase (w/w) in hanging drop setup. Paratone used as cryoprotectant. Another SeMet crystal grown in 16% PEG3350, 0.2M KCl, pH 6.0 with 1:100 Dispase (w/w) in hanging drop setup was used for data refinement. Paratone used as cryoprotectant.

Native crystal used for structure determination was grown in 20% PEG3350, 0.2M KCl with 1:100 Chymotrypsin (w/w) in sitting drop setup. Cryoprotectant used 0.8v well solution + 0.2V 80% Glycerol.
Crystals grow to a mountable size within one week.
NMR Spectroscopy:
Data Collection:
Data Processing: