SEPT2

PDB:2QNR

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:NP_004395
Entry Clone Source: Gene Script
SGC Clone Accession:
Tag:N-terminal hexa histiden tag with thrombin cleavage site: mgsshhhhhhssglvprgs
Host:E.coli BL21 (DE3) codon plus RIL

Construct

Prelude:
Sequence:

gsNLPNQVHRKSVKKGFEFTLMVVGESGLGKSTLINSLFLTDLYPERVISGAAEKIERTVQIEASTVEIEERGVKLRLTVVDTPGYG
DAINCRDCFKTIISYIDEQFERYLHDESGLNRRHIIDNRVHCCFYFISPFGHGLKPLDVAFMKAIHNKVNIVPVIAKADTLTLKERE
RLKKRILDEIEEHNIKIYHLPDAESDEDEDFKEQTRLLKASIPFSVVGSNQLIEAKGKKVRGRLYPWGVVEVENPEHNDFLKLRTML
ITHMQDLQEVTQDLHYENFRSERLKRGGRK

Vector:p28a-LIC

Growth

Medium:TB
Antibiotics:
Procedure: The target 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 chloramphenicol at 37ºC. When OD600 was ~3.0, the culture was induced with 1mM IPTG and the temperature was reduced to 15°C, and the cells were allowed to grow overnight before harvesting and flash frozen.

Purification

Procedure
The thawed cell pellets were suspended in 100 mL of the binding buffer (10 mM Tris pH 7.5, 0.5 M NaCl, 5% glycerol, 5 mM imidazole) with a protease inhibitor cocktail (0.1 mM M benzamidine-HCl and 0.1 mM phenylmethyl sulfonyl fluoride), and 0.5% CHAPS. The cells were lysed by microfluidizer. The lysate was centrifuged at 15000 rpm for 30 min and the supernatant was passed through DE52 (Whatman) column equilibrated with the binding buffer and then loaded onto 3 mL Ni-NTA column (Qiagen) equilibrated with the same binding buffer at

4 ºC. The Ni-NTA column was washed with 150 mL of the wash buffer (10mM Tris pH 7.5, 0.5 M NaCl, 5% glycerol, 30 mM imidazole) and the protein was eluted with 15 mL of the elution buffer (10mM Tris pH 7.5, 0.5 M NaCl, 5% glycerol, 250 mM imidazole). The protein were further purified and desalted using gel filtration column, Superdex 200 (26/60), which was pre-equilibrated with 10 mM Tris pH 8.0, 0.5 M NaCl, 5 mM MgCl2, and 10 mM DTT. All proteins were concentrated using an Amicon Ultra centrifugal filter to a final concentration of 30 mg/mL after the addition of 5mM GDP. Protein concentrations were measured using Bradford assay with purity >95% based on SDS-PAGE analysis.

Extraction

Procedure
The culture was harvested by centrifugation. Pellets from 4 L of culture were resuspended to approximately 40 mL/L of cell culture in Binding Buffer with the addition of protease inhibitors (1 mM benzamidine and 1 mM phenylmethyl sulfonyl fluoride (PMSF)). Resuspended pellets stored at -80 degC were thawed overnight at 4 degC on the day before purification. Prior to mechanical lysis, each pellet from 1 L of culture was pretreated with 0.5 % CHAPS and 500 units of benzonase for 40 minutes at room temperature. Cells were lysed by sonicating for 10min and was centrifuged using a Beckman JA-16.25 rotor at 15,500 rpms for 45 minutes at 4 degC.
Concentration:30 mg/mL
Ligand
GDP**MassSpec:**
Crystallization: Crystallization trials were set up using the sitting drop vapor diffusion method. The protein drop was equilibrated against a reservoir solution (1:1 volume ratio) containing 20% PEG3350 and 0.2M tri-Li citrate. Crystals reached a size of about 100 microns within two to three days.
NMR Spectroscopy:
Data Collection:
Data Processing: