FLJ20628

PDB:2B25

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:GI:47271406
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal: His-tag with integrated thrombin protease site: MGSSHHHHHHSSGLVPRGS
Host:E.coli BL21 (DE3) codon plus RIL (Stratagen).

Construct

Prelude:
Sequence:

gsSTSRERPFQAGELILAETGEGETKFKKLFRLNNFGLLNSNWGAVPFGKIVGKFPGQILRSSFGKQYMLRRPALEDYVVLMKRGTA
ITFPKDINMILSMMDINPGDTVLEAGSGSGGMSLFLSKAVGSQGRVISFEVRKDHHDLAKKNYKHWRDSWKLSHVEEWPDNVDFIHK
DISGATEDIKSLTFDAVALDMLNPHVTLPVFYPHLKHGGVCAVYVVNITQVIELLDGIRTCELALSCEKISEVIVRDWLVCLAKQKN
GILAQKVESKINTDVQLDSQEKIGVKGELFQEDDHEESHSDFPYGSFPYVARPVHWQPGHTAFLVKLRKVKPQLN

Vector:p28a-LIC

Growth

Medium:
Antibiotics:
Procedure:FLJ20628 was expressed in E.coli BL21 (DE3) codon plus RIL in M9 minimal medium in the presence of 50 µg/mL of kanamycin. Cell were grown at 37oC to an OD600 of 0.8 and induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 1 mM, in the presence of 50 mg/L of SeMet and incubated overnight at 15oC.

Purification

Procedure

Extraction

Procedure
Cells were harvested by centrifugation at 7,000 rpm. The cell pellets were frozen in liquid

nitrogen and stored at -80°C. For the purification the cell paste was thawed and resuspended in lysis buffer (20 mM Tris, pH 8.5, 0.5 M NaCl, 5 mM imidazol, 2 mM ß-mercaptoethanol, 5% glycerol, 0.1% CHAPS) with protease inhibitor (0.1mM phenylmethyl sulfonyl fluoride, PMSF). The cells were lysed by passing through Microfluidizer (Microfluidics Corp.) at 20,000 psi.
Concentration:
Ligand
MassSpec:
Crystallization: Purified FLJ20628 was complexed with S-adenosyl-L-methionine (SAM, Sigma) at 1:10 molar ratio of protein: SAM and crystallized using hanging drop vapor diffusion method drop at 20 °C by mixing 1.5 µl of the protein solution with 1.5 µl of the reservoir solution containing 11% PEG3350, 0.2M Li Citrate, 0.1 M BisTris pH 6.5, 10% PEG400.
NMR Spectroscopy:
Data Collection:
Data Processing: