SMAP1L (LOC64744)

PDB:2IQJ

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:
Entry Clone Source:MGC clone AU80-G5
SGC Clone Accession:
Tag:N-terminal hexahistidine tag with thrombin cleavage site: mgsshhhhhhssglvprgs
Host:E.coli BL21 (DE3) codon plus RIL

Construct

Prelude:
Sequence:

gsMTGKSVKDVDRYQAVLANLLLEEDNKFCADCQSKGPRWASWNIGVFICIRCAGIHRNLGVHISRVKSVNLDQWTQEQIQCMQEMG
NGKANRLYEAYLPETFRRPQIDPAVEGFIRDKYEKKKYMDRSLDINA

Vector:pET28a-LIC

Growth

Medium: Terrific Broth
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 resuspended in 100 mL of the binding buffer (20 mM HEPES pH 7.5, 150 mM NaCl, 1 mM mercaptoethanol (BME)) with a protease inhibitor cocktail (0.1 mM M benzamidine-HCl, 0.1 mM phenylmethyl sulfonyl fluoride, and 2uL benzonase [Sigma]), and 0.5% CHAPS. The cells were further lysed by sonication. The lysate was centrifuged at 16000 rpm for 60 min and the supernatant was loaded onto 5 mL HiTrap Ni-NTA column (Pharmacia Amersham) equilibrated with the same binding buffer at 4 ºC using peristaltic pump. Then the Ni-NTA column was connected to FPLC instrument and washed with 25 mL of the wash buffer (20mM HEPES pH 7.5, 150 mM NaCl, 50 mM imidazole, 1mM BME). A linear gradient of

50mL from 50 to 500mM imidazole concentration was then applied and the protein was eluted at around 175mM imidazole concentration. Collected fraction were digested with thrombin (Sigma, ~ 7U/mg protein) overnight at 4 ºC and then passed through an open column filled with ~ 3mL Ni-NTA resin (Qiagen). The flowthrough was collected and supplemented with 200?L PMSF (100mM stock solution) to inhibit residual thrombin protease activity. The protein were further purified and desalted using gel filtration column, Superdex 75 (26/60), which was pre-equilibrated with the binding buffer. Collected fractions were concentrated using an Amicon Ultra centrifugal filter to a final concentration of around 50 mg/mL. Protein concentration was measured using Bradford assay and the purity was greater than 95% based on SDS-PAGE analysis.

Extraction

Procedure

Concentration:30 mg/mL
Ligand
MassSpec:14945.10, expected 15430.63, DNA sequencing confirmed construct design, unclear about the reason of difference.
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 25.0% P3350/0.2M NH4Ac/0.1M Bis-Tris pH6.5. Crystals reached a size of about 50 microns within two to three days.
NMR Spectroscopy:
Data Collection:
Data Processing: