SIAH2

PDB:5H9M
Entry Clone Accession:BC013082
Entry Clone Source:AT13-G11
SGC Clone Accession:YTC020-H07
Tag:N-terminal SUMO tag, removed
Host:BL21-CodonPlus-RIL
Prelude:SIAH2:A131-T321:SUMO
Vector:pET28-MKH8SUMO

Sequence:

gASAVLFPCKYATTGCSLTLHHTEKPEHEDICEYRPYSCPCPGASCKWQGSLEAVMSHLMHAHKSITTLQGEDIVFLATDINLPGAV
DWVMMQSCFGHHFMLVLEKQEKYEGHQQFFAIVLLIGTRKQAENFAYRLELNGNRRRLTWEATPRSIHDGVAAAIMNSDCLVFDTAI
AHLFADNGNLGINVTIST

Growth

Procedure: LEX Bubbling

For protein: The target protein was expressed in E. coli by inoculating 30 mL of overnight culture grown in Luria-Bertani medium into a 1 L of TerrificBroth medium in the presence of 50 ug/mL kanamycin and 34 ug/mLchloramphenicol at 37 degree. When the OD600 of the culture reached ~1.5, the temperature was lowered to 18 degree and theculture was induced with 0.25 mM IPTG. The cells were allowed to growovernight before harvested by centrifugation (7,000 rpm Beckman JLA-8.1000rotor 12min) and flash frozen in liquid nitrogen and stored at -80 degree.

Purification

Procedure: The lysate was centrifuged at 16,000 rpm (25,800xg RCF(average) for 60 minutes and transfered the supernatant to equilibrated 6 mL Ni-NTA resin and incubated for 1 hour at 4 degree, then loaded them to Bio-rad open gravity column. The beads in theopen coumn was then washed with 25 mL lysis buffer followed by with 50 mL washing

buffer. Bound proteins were eluted using 15 mL elution buffer. The N-terminal Sumo-tag was reomoved by overnight incubation with TEV protease (1:30 w/w) at 4degree during dialysis against the dialysis buffer. Uncut proteins and TEVprotease were removed by passing the solution through 3mL Ni-NTA beads,andthe target protein was further purified by anion-exchange chromatography on 5mL HiTrap S column(GE Healthcare). Then the protein was further purified bygel filtration on a HighLoad 16/60 Superdex 200 column (GE Healthcare)preequilibrated with gel filtration buffer. Fractions containing targetprotein were pooled and concentrated by centrifugal filters (Amicon mwco10kDa). The final yield of the protein was about 1.3 mg per litre bacterialculture and the purity is above 99% judging from SDS-PAGE.

Extraction

Procedure: 4L cell pellet was resuspended in a total volume of 400 ml lysis buffer with1mM PMSF/Benzamidine freshly added and the cells disrupted by sonication for 10 mins at 5" on 7" off duty cycle at 120W output power,

Concentration:11.15 mg/ml

Structure Determination

MassSpec:native protein without SUMO tag measured: 21354.7 g/mol, expected 21354.4 g/mol.
Crystallization: The crystals were grown at 298K using the sitting drop method by mixing 0.5 uL protein with 0.5 uL well solution consisting of 25%PEG8000, 0.2 M NaCl, 0.1 M Hepes pH 7.5.The crystals were cryoprotected by immersion in well solution containing 15% ethylene glycol firstly, then immersion in Paratone.