SETD8

PDB:4IJ8

Revision

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Entry Clone Accession:BC050346
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SGC Clone Accession:SETD8:PBC001-C06:C218639
Tag:N-terminal His6-tag, removed by TEV
Host:BL21-V2R-pRARE

Construct

Prelude: Template was obtained from Raymond C. Trievel in University of Michigan. The construct contains a mutation C302S
Sequence:

AMGSRKSKAELQSEERKRIDELIESGKEEGMKIDLIDGKGRGVIATKQFSRGDFVVEYHGDLIEITDAKKREALYAQDPSTGCYMYY
FQYLSKTYCVDATRETNRLGRLINHSKSGNCQTKLHDIDGVPHLILIASRDIAAGEELLYDYGDRSKASIEAHPWLKH

Vector:pHIS2

Growth

Medium:
Antibiotics:
Procedure:LEX Bubbling. The target protein was expressed in E. coli by inoculating 30 mL of overnight culture grown in Luria-Bertani medium into a 2 L of Terrific Broth medium in the presence of 50 ug/mL ampicillin and chloramphenicol at 37 degree. When OD600 reached ~2.0, the temperature of the medium was lowered to 15 degree and the culutre was induced with 0.5 mM IPTG. The cells were allowed to grow overnight before harvested by centrifugation (7,000 rpm 15min) and flash frozen in liquid nitrogen and stored at -80 degree.

Purification

Procedure
The protein was purified by purified to homogeneity using Ni-NTA affinity and Superdex 75 (Amersham Biosciences) gel filtration chromatographies in 20 mM Tris-HCl at pH 7.0, 100 mM NaCl, and 5 mM DTT, the his tag was removed by TEV before gel filtration.

Extraction

Procedure
2L cell pellet was resuspended in a total volume of 200 ml lysis buffer and the cells disrupted by sonication using Microfluidizer (Microfluidics M110-EH).
Concentration:20.0 mg/mL
Ligand
SAM**MassSpec:**The cut version native protein expected 18743.1, measured 18743.1
Crystallization: Crystals were obtained at 18 °C using the vapor diffusion method by mixing a protein solution at a concentration of 18 mg/mL (in 20 mM Tris-HCl pH 7.0-8.5,100 mM NaCl, 5 mM DTT) with a 10-fold excess of SAM with an equal volume of reservoir solution (1.08-1.2 M Trisodium citrate, 100 mM HEPES, pH 7.5).
NMR Spectroscopy:
Data Collection:
Data Processing: