DOT1L

PDB:4EQZ

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession: GenBank: AF509504.1
Entry Clone Source:DNA 04-G3 (GeneScript)
SGC Clone Accession:DOT1L:JMC01M-E09:C206422
Tag:N-terminal His6-tag, removed by TEV
Host:BL21-V2R-pRARE

Construct

Prelude:sythetic gene
Sequence:

MGEKLELRLKSPVGAEPAVYPWPLPVYDKHHDAAHEIIETIRWVCEEIPDLKLAMENYVLIDYDTKSFESMQRLCDKYNRAIDSIHQ
LWKGTTQPMKLNTRPSTGLLRHILQQVYNHSVTDPEKLNNYEPFSPEVYGETSFDLVAQMIDEIKMTDDDLFVDLGSGVGQVVLQVA
AATNCKHHYGVEKADIPAKYAETMDREFRKWMKWYGKKHAEYTLERGDFLSEEWRERIANTSVIFVNNFAFGPEVDHQLKERFANMK
EGGRIVSSKPFAPLNFRINSRNLSDIGTIMRVVELSPLKGSVSWTGKPVSYYLHTIDRTILENYFSSLKNPKLREEQEAARRRQQRE
SKSNAATPTKGPEGKVAGPADAPMDSGAEEEKAGAATVKKPSPSKARKKKLNKKGRKMAGRKRGRPKKMNTA

Vector:pET28-MHL

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 kanamycin and chloramphenicol at 37 °C . When OD600 reached ~2.0, the temperature of the medium was lowered to 15 °C 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 °C .

Purification

Procedure
DOT1L (1-420) was purified by Ni-NTA column (Qiagen) and processed by in-house produced TEV protease to remove the His tag. The protein was then incubated in 50 mM Tris-HCl pH 8.0, 1 mM MgCl2 with benzonase nuclease for 2 hours at room temperature to remove DNA which binds to the C-terminal region of DOT1L (1-420). The filtered protein sample was diluted with

50 mM K2HPO4/ KH2PO4 pH 7.0, and further purified by HiTrap-SP (GE Healthcare). The protein was finally purified by gel filtration (Superdex 200, GE Healthcare)

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:16.0 mg/mL
Ligand
FED2**MassSpec:**The cut version native protein expected 47902.6, measured 47902.6
Crystallization: The crystals obtained by soaking compound into crystals that generate structure of 3UWP (DOT1L/5iodotubercidin, material and methods available by referring to that of 3UWP). For displacement soaking, solid compound FED2 first dissolved in Milli-Q water to obtain 10 mM aqueous stock solutions. Compound solutions for displacement soaking were prepared by diluting 1 µL stock solution of each compound with 19 µL reservoir buffer to make 0.5 mM compound solutions. Crystals of DOT1L (1-420)/5-iodotubercidin were then transferred into 1.5 µL of each compound solution and incubated for 4-24 hours at 18°C. Prior to being flash-frozen in liquid nitrogen, the crystals were soaked in a cryoprotectant consisting of 80% reservoir solution and 20% glycerol.
NMR Spectroscopy:
Data Collection:
Data Processing: