DOT1L

PDB:3SX0

Revision

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Revised by:created
Revision Date:created
Entry Clone Accession:GI:206422
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SGC Clone Accession:JMC01ME09
Tag:N-terminal: His-tag with integrated TEV protease site: MHHHHHHSSGRENLYFQ*G
Host:E.coli BL21 (DE3) V2RpRARE

Construct

Prelude:
Sequence:

GMGEKLELRLKSPVGAEPAVYPWPLPVYDKHHDAAHEIIETIRWVCEEIPDLKLAMENYVLIDYDTKSFESMQRLCDKYNRAIDSIH
QLWKGTTQPMKLNTRPSTGLLRHILQQVYNHSVTDPEKLNNYE PFSPEVYGET SFDLVAQMID EIKMTDDDLF VDLGSGVGQV
VLQVAAATNCKHHYGVEKADIPAKYAETMDREFRKWMKWYGKKHAEYTLE RGDFLSEEWR ERIANTSVIFVNNFAFGPEV DHQL
KERFAN MKEGGRIVSS KPFAPLNFRI NSRNLSDIGT IMRVVELSPLKGSVSWTGKPVSYYLHTIDRTILENYFSSL KNPKLR
EEQEAARRRQQRES KSNAATPTKGPEGKVAGPADAPMDSGAEEE KAGAATVKKP SPSKARKKKLNKKGRKMAGRKRGRPKKMNT
A

Vector:pET28a-MHL

Growth

Medium: Terrific Broth (TB) medium
Antibiotics:50 µg/ml of kanamycin
Procedure: Growth method DOT1L was expressed in E.coli BL21 (DE3) V2RpRARE in Terrific Broth (TB) medium in the presence of 50 µg/mL of kanamycin. Cell were grown at 37 °C to an OD600 of 1.5 and induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 1 mM, and incubated overnight at 15 °C

Purification

Procedure
The protein was purified by Ni-NTA column (Qiagen) and processed by TEV protease to remove the His tag. The protein was then incubated in 50 mM Tris-HCl pH 8.0, 0.1 mg/ml BSA, 1 mM MgCl2 with benzonase nuclease for 2 hours at room temperature to get rid of the DNA. Filtered protein sample was diluted with 50 mM KiPO4 pH7, and further purified by HiTrap-SP (GE Healthcare). The protein was finally purified by size exclusion chromatography (Superdex 200, GE Healthcare)

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 with protease inhibitor (0.1 mM phenylmethyl sulfonyl fluoride, PMSF). The cells were lysed by sonication.
Concentration:16mg/ml
Ligand
(2S)-2-amino-4-({[(2S,3S,4R,5R)-5-(4-amino- 5-bromo-7H-pyrrolo[2,3-d]pyrimidin-7-yl)- 3,4-dihydroxytetrahydrofuran-2-yl]methyl}sulfanyl)butanoic acid**MassSpec:**Expected MW is 47902.6 Da - Measured MW is 47903 Da
Crystallization: Crystal was initially obtained from RW screen condition D6(3.5 M Sodium Formate, 0.1M Sodium Acetate, pH 4.6), using 0.5 uL protein(mixed with 5 times of compound) + 0.5 uL well solution against 100 uL reservoir buffer at 18 °C. Crystals grow to a mountable size in three days.
NMR Spectroscopy:
Data Collection:
Data Processing: