

DOT1L

PDB:4ER7

Revision

Revision Type:created

Revised by:created

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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:synthetic gene

Sequence:

MGEKLELRKSPVGAEPVYPWPLPVYDKHHDAAEHIIETIRWCEEIPDLKLAMENYVLIDYDTKSFESMQRLCDKYNRAIDSIHQ
LWKGTTQPMKLNTRPSTGLLRHILQQVYNHSVTDPEKLNNYEPFSPEVYGETSFDLVAQMIDEIKMTDDDLFVDLGSGVGQVVLQVA
AATNCKHHYGEKADIPAKYAETMDREFRKWMKWYGKKHAEYTLERGDFLSEEWREIRIANTSVIFVNNFAFGPEVDHQLKERFANMK
EGGRIVSSKPFAPLNFRINSRNLSDIGTIMRVVELSPLKGSVSWTGKPVSYYLHTIDRTILENYFSSLKNPKLREEQEAARRRQRE
SKSNAATPTKGPEGKVAGPADAPMDSGAEEEEKAGAATVKKPSPSKARKKKLNKKGRKMAGRKRGRPKKMNTA

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 culture 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

SGC0947**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 SGC0947 first dissolved in 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: