DOT1L

PDB:4ER3

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:PBC001-F03:C223238
Tag:N-terminal His6-tag, removed by TEV
Host:BL21-V2R-pRARE

Construct

Prelude:sythetic gene
Sequence:

```
MGEKLELRLKSPVGAEPAVYPWPLPVYDKHHDAAHEIIETIRWVCEEIPDLKLAMENYVLIDYDTKSFESMQRLCDKYNRAIDSIHQ
LWKGTTQPMKLNTRPSTGLLRHILQQVYNHSVTDPEKLNNYEPFSPEVYGETSFDLVAQMIDEIKMTDDDLFVDLGSGVGQVVLQVA
AATNCKHHYGVEKADIPAKYAETMDREFRKWMKWYGKKHAEYTLERGDFLSEEWRERIANTSVIFVNNFAFGPEVDHQLKERFANMK
EGGRIVSSKPFAPLNFRINSRNLSDIGTIMRVVELSPLKGSVSWTGKPVSYYLHTIDRTILENYFSSLKNPKLREEQEAARRRQQRE
SKS
```

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
For purification, the cell paste was thawed and resuspended in lysis buffer with 1mM phenylmethyl sulfonyl fluoride (PMSF). DOT1L (1-351) was purified by Ni-NTA column (Qiagen) and processed by in-house produced TEV protease to remove the His tag. 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
EPZ004777**MassSpec:**The cut version native protein expected 40663.2, measured 40663.2
Crystallization: To obtain crystals of DOT1L(1-351)/EPZ004777, crystals of DOT1L(1-351) with SAH were first prepared for displacement soaking. Crystals of DOT1L (1-351) complexed with SAH were obtained at 18 °C using the vapor diffusion method by mixing a protein solution at a concentration of 20 mg/mL (in 20 mM Tris-HCl pH 8.0, 200 mM NaCl, 1 mM EDTA, and 1 mM TCEP) with a 5-fold excess of SAH with an equal volume of reservoir solution (1.6M (NH4)2SO4, 0.01M MgCl2, 0.1M NaCaCo, pH 5.5). Solid EPZ004777 was dissolved in Milli-Q water to obtain a 10 mM stock solution. For soaking, 1 uL of 10 mM EPZ004777 stock solution was mixed with 19 uL of reservoir buffer to prepare 0.5 mM EPZ004777 in soaking buffer. Crystals of DOT1L(1-351)/SAH were transferred into 1.5 uL soaking buffer and incubated for 12 hours during which time EPZ004777 displaced SAH in the crystals.
NMR Spectroscopy:
Data Collection:
Data Processing: