SGC Clone Accession:JMC76B5

Tag:N-terminal hexahistidine tag

Host:E. Coli BL21 (DE3)-V2R-pRARE2

Construct
DOT1L Sequence:
TYEDLVQAQKEITAHNMQLREQTKQLEHDMAELRDQSQLLLKARCEELK
Vector: pET28-MKH8SUMO

AF10: Sequence:
SSLENLPPVAASIEQLLERQWSEGQQFLLEQGTPSDILGMLKSLHQLQVENRRLEEQIKNL
TAKKERLQLLNAQLS

Vector:p28a-MHL

Growth
Medium:TB
Antibiotics: Kan
Procedure:A glycerol stock was used to inoculate 20 mL LB media containing 50 µg/mL kanamycin. The culture was grown overnight at 37°C with shaking. The next day this starter culture was used to inoculate 2L of TB medium which contained kanamycin. The culture was grown in LEX at 37°C to OD600 ~3.0 and was induced with the addition of 0.5 mM IPTG. The temperature was reduced to 16°C and the culture was incubated for a further 16 hours before harvesting the cells.

Extraction Procedure
cells were harvested by centrifugation and pellets were stored in -80ºC. Prior to purification, the cell pellet was resuspended in lysis buffer. The cells were homogenised and sonicated on ice with 10 second pulses and 30 second rest intervals. The output level was 8.5 for 6 mins. The sample was centrifuge at 16,000rpm for 1 hour. The supernatant was purified by affinity, size exclusion and ion exchange.
Concentration:26 mg/ml.

Purification Procedure

For the DOT1L-AF10complex, the pellets of DOT1Lwere mixed with that of AF10O to sonicate. The complex proteins were first purified by nickel affinity chromatography and further purified using Mono-Q and Superdex 200, eluted with buffer C (25 mM Tris-HCl, pH 8.0, 150 mM NaCl). Peak fractions were concentrated to 26 mg/ml. The fractions containing protein were identified on a SDS-PAGE gel.

Crystallization: 0.1 M sodium acetate (pH 4.6), 0.2 M calcium chloride, 0.01 M zinc chloride, and 30% (w/v) MPD