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Certificate of Analysis

DOT1L

Human Disruptor of Telomeric silencing 1-like, active

(Recombinant enzyme expressed in *E.coli*)
Item # EPI018
Lot # 139683

Product Description: Human recombinant DOT1L, amino acids 1-420, expressed in *E.coli*. Purified using immobilised metal affinity chromatography.
MW = 47.9kDa.

Tag cleaved by TEV.

Aliases : KMT4, KIAA1814

Formulation: 1mg/ml of enzyme in 25mM Hepes/NaOH pH7.4, 250mM NaCl, 50% Glycerol, 2mM DTT. Frozen solution.

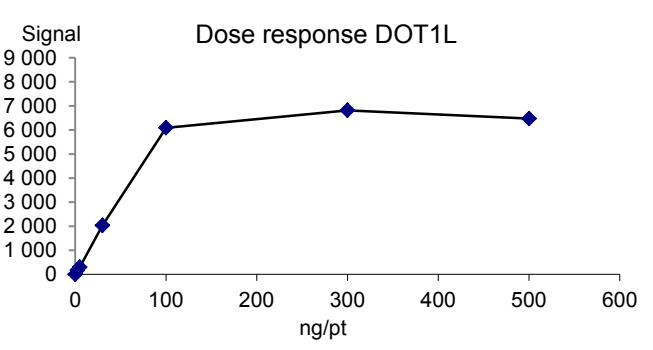
Storage and Stability: Stable for 1 year at -70°C from date of shipment. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled microcentrifuge tubes and store at -70°C.

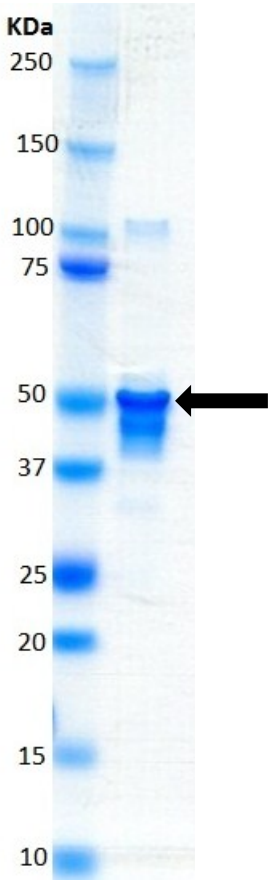
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

HMT Assay: 2.5-500ng of this lot of enzyme transferred methyl groups from [3H] SAM to HeLa Polynucleosome in the assay described on page two. Assay background was subtracted from the actual counts to yield the results shown below.



MS: Size was confirmed by mass spectrometry using a Q-TOF.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 4µg of DOT1L.

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DOT1L Assay Protocol

Stock Solutions:

1. **Reaction buffer:** 20 mM Tris/HCl pH8.0, 100mM NaCl, 2mM DTT.
2. **DOT1L, active:** Dilute with reaction buffer. Use 2.5-500ng per assay point.
3. **HeLa Polynucleosome:** Dilute with reaction buffer to 12.5µg/ml.
4. **[3H] SAM:** Dilute with reaction buffer to 200nM.
5. **Filtration Buffer :** 33mM Citric acid pH2.2

Assay Procedure (96 well plate format):

1. Add 5µl of 10% DMSO per assay to each well.
2. Add 25µl of [3H] SAM.
3. Add 10µl **(2.5-500ng) DOT1L, active**.
4. Add 10µl of HeLa Polynucleosome.
5. Incubate for 15 minutes at 22°C.
6. Stop the reaction by adding 500µl of citric acid, then filter on a GF/B Filter. Wash 3 times with filtration buffer.
7. Dry and add scintillation cocktail.
8. Read in a scintillation counter. Compare the signal of enzyme samples with that of a background sample that contains all assay components except the enzyme DOT1L.

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DOT1L Sequence Information

Protein Human DOT1L

Tags *Tag cleaved by TEV*

Accession number GenBank NP_115871.1

Recombinant DOT1L amino acid sequence:

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  1 GMGEKLELRL KSPVGAEPAV YPWPLPVYDK HHDAAHEIIE TIRWVCEEIP
 51 DLKLAMENYV LIDYDTKSFE SMQRLCDKYN RAIDSIHQLW KGTTQPMKLN
101 TRPSTGLLRH ILQQVYNHSV TDPEKLNNYE PFSPEVYGET SFDLVAQMID
151 EIKMTDDDLF VDLGSGVGQV VLQVAAATNC KHHYGVEKAD IPAKYAETMD
201 REFRKWMKWY GKKHAEYTLE RGDFLSEEWR ERIANTSVIF VNNFAFGPEV
251 DHQLKERFAN MKEGGRIVSS KPFAPLNFRI NSRNLSDIGT IMRVVELSPL
301 KGSVSWTGKP VSYYLHTIDR TILENYFSSL KNPKLREEQE AARRRQQRES
351 KSNAATPTKG PEGKVAGPAD APMDSGAEEE KAGAATVKKP SPSKARKKKL
401 NKKGRKMAGR KRGRPKKMNT A
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Reviewed and approved by site quality representative.

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