REACTION BIOLOGY

PRODUCT DATASHEET

DOT1L (DOT1-like, histone H3 methyltransferase; H3-K79-HMTase; KMT4)

CATALOG NO.: HMT-11-101 **LOT NO.:**

DESCRIPTION: Human recombinant DOT1L (residues 1-416; Genbank Accession # NM_032482) expressed as an N-terminal GST-fusion protein in *E. coli.* MW = 80.0 kDa. Catalyzes the transfer of methyl groups from S-adenosyl-L-methionine (SAM) to the ε-amino function of protein L-lysine residues, specifically lysine-79 of histone H3 (H3K79). Human DOT1L, and its homologs in other species (e.g. founding exemplar yeast DOT1), are unusual among the histone lysine methyltransferases in that they are specific for a residue (H3K79) that occurs in the histone H3 globular domain, rather than its N-terminal tail[1,2]. Further, uniquely among the lysine methyltransferases, members of the DOT1 family do not contain a SET domain[1,2]. Although essential to telomeric silencing[1] and heterochromatin formation[3], DOT1L and H3K79 methylation are also associated with the activation of gene transcription[4,5]. Inappropriate recruitment of DOT1L by MLL-fusion proteins is implicated in the development of leukemias[6-8].

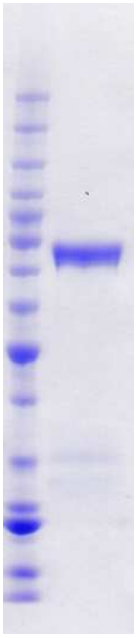
PURITY: >90% by SDS-PAGE.

ASSAY CONDITIONS: RBC's DOT1L displays histone methyltransferase activity with HeLa oligo or mono/di-nucleosomes (0.05 mg/mL as [DNA])) and to a far lesser degree (~100x) with chicken core histones (0.05 mg/mL), as TCA-precipitated counts in a scintillation/filter plate assay (Multiscreen FB, Topcount). Reaction conditions are: 50 mM Tris-HCl, pH 8.5, 50 mM NaCl, 5 mM $MgCl_2$, 1 mM DTT, 1 mM PMSF, 30°C with substrates at concentrations indicated above. (See figure, below.)

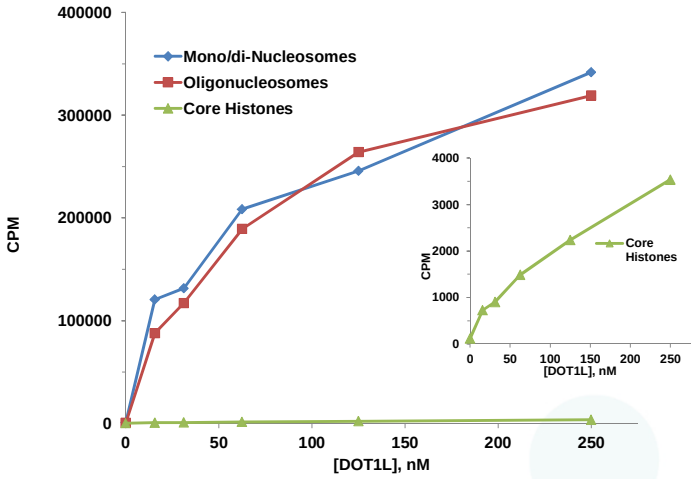
SUPPLIED AS: __ µg/µl in 25 mM Tris-HCl, pH 7.5, 100 mM NaCl, 0.05% Tween 20, 3 mM DTT, 20% (v/v) glycerol as determined by OD_{280}.

STORAGE: -70°C. Thaw quickly and store on ice before use. The remaining, unused, undiluted enzyme should be refrozen quickly by, for example, snap freezing in a dry/ice ethanol bath or liquid nitrogen. Freezing and storage of diluted enzyme is not recommended.

REFERENCES: 1) H.H. Ng *et al. Genes Dev.* 2002 **16** 1518; 2) Q. Feng *et al. Curr. Biol.* 2002 **12** 1052; 3) B. Jones *et al. PLOS Genet.* 2008 **4** e1000190; 4) D. Schubeler *et al. Genes Dev.* 2004 **18** 1263; 5) D. Steger *et al. Mol. Cell. Biol.* 2008 **28** 2825; 6) A.V. Krivtsov *et al. Nat. Rev. Cancer* 2007 **7** 823; 7) A.V. Krivtsov *et al. Cancer Cell* 2008 **14** 55; 8) M. Guenther *et al. Genes Dev.* 2008 **22** 3403



Coomassie blue stained SDS-PAGE (4-12% acrylamide) of 4 µg of purified RBC DOT1L. MW markers (left) are, from top, 220, 160, 120, 100, 90, 80, 70, 60, **50**, 40, 30, 25, **20**, 15, 10 kDa.



Methylation Activity of Dot1L with HeLa Mono/Di-Nucleosomes, Oligonucleosomes and Chicken Core Histones. Assays were performed with a scintillation/filter plate assay. Incubations were 60 min., 30°C with HeLa mono/di-nucleosomes (RBC Cat. # HMT-35-123) or HeLa oligonucleosomes (RBC Cat. # HMT-35-130), both 0.05 mg/mL as [DNA] or chicken core histones (0.05 mg/mL) and 1 µM [^{3}H]-SAM. See inset for core histone data on 100x expanded scale.

This product is not intended for therapeutic or diagnostic use in animals or in humans.

Reaction Biology
1 Great Valley Parkway, Malvern PA, USA 19355
requests@reactionbiology.com www.reactionbiology.com