PRODUCT DATASHEET

ASH1L (Ash1-Like Protein)

CATALOG NO.: HMT-11-376 **LOT NO.:**

DESCRIPTION: Human recombinant ASH1L (residues 2046-2330; Genbank Accession # NM_018489; MW = 35.4 kDa) expressed in *E. coli* with an N-terminal His-tag. Catalyzes the transfer of methyl groups from S-adenosyl-L-methionine (SAM) to the ε-amino function of protein L-lysine residues, specifically lysine-36 of histone H3 (H3K36)[1,2], a mark associated with active transcription. Activity has also been reported at H3K4[3]. A large, multi-domain protein associated with actively transcribed regions of chromatin, ASH1L is the human homolog of *Drosophila* Ash1, a Trithorax group protein. Like its counterpart in *Drosophila*[4], ASH1L contains a SET histone methyltransferase domain and has been found to play a role in the regulation of Hox gene expression[3,5]. Although the ASH1L SET domain has been shown *in vitro* to methylate histone peptides on lysine-4 of histone H3 (H3K4)[3], *in vivo* or *in vitro* with nucleosomes as substrate, ASH1L is an H3K36 methyltransferase[1,2,6]. Recruitment of ASH1L by the ncRNA *DBE-T* to the chromosome 4q35 locus associated with FSHD (facioscapulohumeral muscular dystrophy) leads to increased H3K36me2 and inappropriate gene derepression at the FSHD locus[6]. In a possible positive feedback loop, ASH1L increases expression of *DBE-T* itself[6], suggesting ASH1L's methyltransferase activity or its interaction with *DBE-T* as potential therapeutic targets for FSHD. RBC's ASH1L comprises the catalytic domain (AWS/SET/Post-SET) fused to 6xHis-tag.

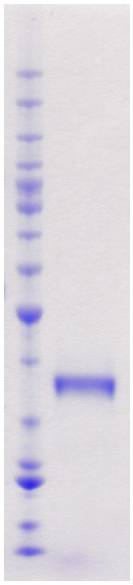
PURITY: >80% by SDS-PAGE

ASSAY CONDITIONS: RBC's ASH1L displays histone methyltransferase activity at concentrations of 60 nM and 100nM, 60 min. reactions, 30°C, as TCA-precipitated counts in a scintillation/filter plate assay (Multiscreen FB, Topcount), with HeLa oligo or mono/di-nucleosomes , Chicken oligo or mono/di nucleosomes and recombinant tetra or mononucleosomes (0.05 mg/mL as [DNA])). Reaction conditions are: 20 mM Tris, pH 9.0, 1 mM TCEP, 0.01% Triton X-100, 1.25mM $MgCl_2$, substrates at concentrations indicated above.

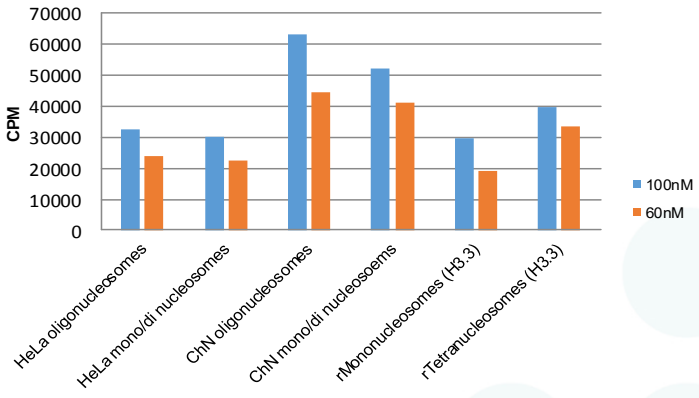
SUPPLIED AS: ___ µg/µL in 100 mM HEPES, pH 7.1, 500 mM NaCl, 0.5 mM TCEP, 5% (v/v) glycerol

STORAGE: -70°C. Thaw quickly and store on ice before use. The remaining, unused, undiluted enzyme should be snap frozen, for example in a dry/ice ethanol bath or liquid nitrogen. Minimize freeze/thaws if possible, but very low volume aliquots (<5 µl) or storage of diluted enzyme is not recommended.

REFERENCES: 1) Y Tanaka *et al. Gene* 2007 **397** 161; 2) S. An *et al. J. Biol. Chem.* 2011 **286** 8369; 3) G. Gregory & *et al. Mol. Cell. Biol.* 2007 **27** 8466;4) C. Beisel *et al. Nature* 2002 **419** 857; 5) Y Tanaka *et al. PLOS One* 2011 **6** e28171; 6) D.S. Cabianca *et al. Cell* 2012 **149** 819;



Coomassie blue stained SDS-PAGE (4-20% acrylamide) of 2 µg of RBC ASH1L. 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 ASH1L with various types of nucleosomes. Assays were performed with a scintillation/filter plate assay. 100nM or 60nM ASH1L was incubated 60 min., 30°C with HeLa Oligonucleosomes (Cat. #HMT-35-130), HeLa Mononucleosome(Cat. # HMT-35-123), Chicken Oligonucleosomes (Cat. #HMT-35-177), Chicken Mononucleosomes (Cat. #HMT-35-179), Recombinant Tetranucleosomes (Cat. #HMT-15-367) and Recombinant Mononucleosomes (Cat. #HMT-15-369); 0.05 mg/mL as [DNA]) and 3.25 µM SAM (1:1.6[^{3}H]-SAM:unlabeled).

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