

EZH1 Complex (Enhancer of Zeste Homolog 1 in complex with AEBP2, EED, RbAp48 & SUZ12)

CATALOG NO.: HMT-25-115

LOT NO.:

DESCRIPTION: Human recombinant EZH1 (residues 2-747; Genbank Accession # NM_001991; MW = 88.4 kDa) in complex with human recombinants AEBP2 (2-517; NM_001114176; 57.7 kDa), EED (2-441; NM_003797; 51.2 kDa), RbAp48 (2-425; NM_005610; 50.9 kDa) and SUZ12 (2-739; NM_015355; 86.3 kDa). Total complex MW is 334.5 kDa. All proteins are full-length (residue 2 through C-terminus) and co-expressed in an insect cell/baculovirus expression system. The EED subunit incorporates an N-terminal Flag-tag and all others include 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-27 of histone H3 (H3K27). During development, Polycomb Repressive Complex 2 (PRC2) is the principal methyltransferase responsible for generating trimethylated histone H3 lysine-27 (H3K27me3), an epigenetic mark essential for programmed repression of gene expression¹⁻⁵. EZH2, which includes a SET methyltransferase domain, functions as the catalytic subunit of PRC2^{1,6}, but can be replaced by the homologous EZH1⁷, which is less catalytically active^{8,9}, although PRC2-Ezh1 has strong repressive activity on transcription independent of its methyltransferase activity⁹. EZH1 acts to preserve the repressive H3K27me3 mark in embryonic stem cells, complementing the role of EZH2⁸. Multiple recent results suggest important roles for EZH1 in developmental and homeostatic processes, including maintenance of the left-right axis¹⁰, hair follicle homeostasis and wound repair¹¹, skeletal muscle differentiation¹² and adult hematopoiesis¹³.

PURITY: >90% by SDS-PAGE.

ASSAY CONDITIONS: RBC's EZH1 Complex displays histone methyltransferase activity at enzyme concentrations of 15.6 nM and above, 30°C, with chicken core histones (0.05 mg/mL) or recombinant histone H3 (1 μM) as TCA-precipitated counts in a scintillation/filter plate assay (Multiscreen FB, Topcount). Reaction conditions are: 50 mM Tris-HCl, pH 8.0, 50 mM NaCl, 1 mM EDTA, 1 mM DTT, 1 mM PMSF, with substrates at concentrations indicated above and [³H]-SAM.

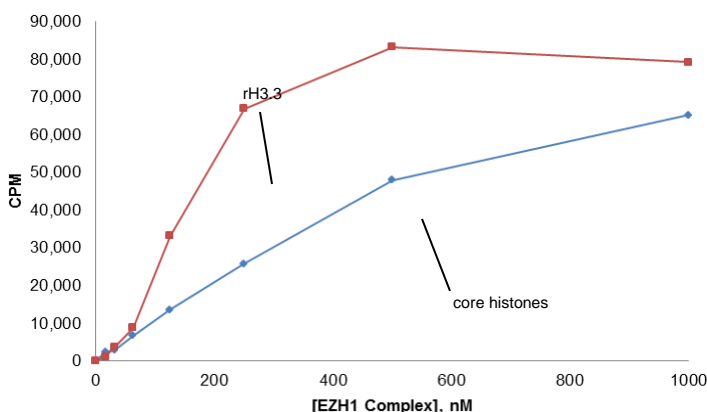
SUPPLIED AS: ___ μM EZH1 Complex, as defined above, (___ μg/μl total protein) in 20 mM Tris-HCl, pH 7.9, 150 mM NaCl, 2 mM MgCl₂, 2 mM DTT, 20% glycerol (w/v), 0.01% NP-40 as determined by OD₂₈₀.

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) R. Cao *et al. Science* 2002 **298** 1039; 2) K. Plath *et al. Science* 2003 **300** 131; 3) J. Silva *et al. Dev. Cell* 2003 **4** 481; 4) S. Erhardt *et al. Development* 2003 **130** 4235; 5) R. Cao & Y. Zhang *Curr. Opin. Genet. Dev.* 2004 **14** 155; 6) R. Cao & Y. Zhang *Mol. Cell* 2004 **15** 57; 7) K.J. Abel *et al. Genomics* 1996 **37** 161; 8) X. Shen *et al. Mol. Cell* 2008 **32** 491; 9) R. Margueron *et al. Mol. Cell* 2008 **32** 503; 10) D. Arai *et al. Dev. Biol.* 2010 **341** 459; 11) E. Ezhkova *et al. Genes Dev.* 2011 **25** 485; 12) L. Stojic *et al. Epigenetics Chromatin* 2011 **4** 16; 13) M. Mochizuki-Kashio *et al. Blood* 2011 doi: 10.1182/blood-2011-03-340554



Coomassie blue stained SDS-PAGE (4-12% acrylamide) of 2 μg of the purified EZH1 Complex. MW markers at left, from top: 220, 160, 120, 100, 90, 80, 70, 60, 50, 40, 30, 25, 20, 15, 10 kDa. Positions of components are: EZH1-88.4 kDa, SUZ12-86.3 kDa, AEBP2-57.7 kDa, EED-51.2 kDa, RbAp48-50.9 kDa



Methyltransferase Activity of the EZH1 Complex. Methylation determined as TCA-precipitable counts in a scintillation/filter plate assay. Reactions were 60 min., 30°C, 1 μM [³H]-SAM with 1 μM histone H3 (RBC cat # HMT-11-134) or 0.05 mg/mL core histones.

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