

## EZH2 Trimer Complex

(Enhancer of Zeste Homolog 2 in complex with EED & SUZ12)

CATALOG NO.: HMT-23-501

LOT NO.:

**DESCRIPTION:** Human recombinant EZH2 (residues 2-746; Genbank Accession # NM\_001203247; MW = 88.6 kDa) in complex with human recombinants EED (2-441; NM\_003797; 53.9 kDa) and SUZ12 (2-739; NM\_015355; 86.3 kDa). Total complex MW is 228.7 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 Strep and 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  $\epsilon$ -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 expression<sup>1-5</sup>. EZH2, which includes a SET methyltransferase domain, is the catalytic subunit of PRC2<sup>1,6</sup>. The core of the catalytic complex includes EZH2, EED, SUZ12 and RbAp48, while addition of AEBP2 significantly enhances the methyltransferase activity of the complex (>3x)<sup>6</sup>. EZH2 is overexpressed in a wide range of human cancers and its overexpression can correlate with tumor progression, increased metastasis and poor prognosis (see review<sup>7</sup>). Depletion of EZH2 and/or other PRC2 components can inhibit growth or induce apoptosis in cancer cells<sup>8-10</sup>. Consequently, EZH2 is considered a promising target for the development of anti-cancer therapies<sup>11</sup>.

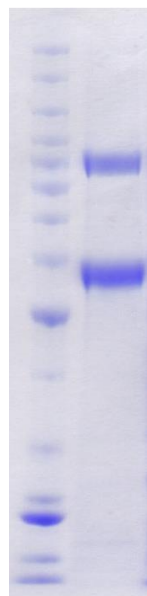
**PURITY:** >90% by SDS-PAGE.

**ASSAY CONDITIONS:** RBC's EZH2 Trimer Complex displays histone methyltransferase activity at enzyme concentrations of 25.6 nM and above, 30°C, with HeLa oligo nucleosomes (0.05mg/mL), chicken core histones (0.05 mg/mL) 1 $\mu$ M rH3.3 histone or 0.5 $\mu$ M recombinant (H3-H4)<sub>2</sub> tetramer 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, 0.01% Brij30, with substrates at concentrations indicated above and [<sup>3</sup>H]-SAM.

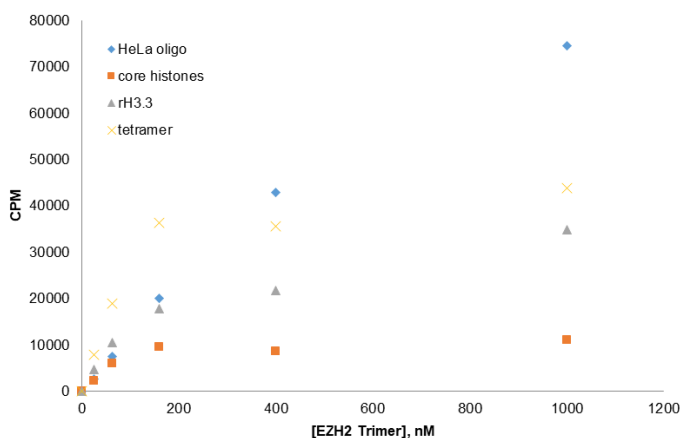
**SUPPLIED AS:** \_\_  $\mu$ M EZH2 Complex, as defined above, (\_\_  $\mu$ g/ $\mu$ l total protein) in 20 mM Tris-HCl, pH 8, 150 mM NaCl, 2 mM MgCl<sub>2</sub>, 2 mM DTT, 20% glycerol (w/v), 0.01% NP-40 as determined by OD<sub>280</sub>.

**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  $\mu$ l) 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) D.P.F. Tsang & A.S.L. Cheng *J. Gastroenterol. Hepatol.* 2011 **26** 19; 8) S. Varambally *et al. Nature* 2002 **419** 624; 9) A.P. Bracken *EMBO J.* 2003 **22** 5323; 10) J. Tan *et al. Genes Dev.* 2007 **21** 1050; 11) R.A. Copeland *et al. Nature Rev. Drug Disc.* 2009 **8** 724



**Coomassie blue stained SDS-PAGE (4-12% acrylamide) of 4  $\mu$ g of the purified EZH2 Trimer 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: EZH2-88 kDa, SUZ12-86 kDa,, EED-53.9 kDa



**Methyltransferase Activity of the EZH2 Trimer Complex.** Methylation determined as TCA-precipitable counts in a scintillation/filter plate assay. Reactions were 60 min., 30°C, 1  $\mu$ M [<sup>3</sup>H]-SAM with 0.05mg/mL HeLa oligonucleosomes, 0.05mg/mL core histones, 1  $\mu$ M histone H3 or 0.5 $\mu$ M tetramer.

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

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