

EZH2-Y641F Complex (Enhancer of Zeste Homolog 2 mutant in complex with AEBP2, EED, RbAp48 & SUZ12)

**CATALOG NO.:** HMT-25-173

**LOT NO.:**

**DESCRIPTION:** Mutant human recombinant EZH2 with phenylalanine (F) substituted for tyrosine-641 (Y641) (otherwise contains wild-type residues 2-746; Genbank Accession # NM\_001203247; MW = 88.6 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 334.7 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 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 mutations at Y641, including Y641F, are associated with certain types of lymphoma<sup>7</sup> in which they occur as heterozygotes with the wild-type allele<sup>7,8</sup>. While wild-type EZH2 is most efficient at monomethylation of unmethylated H3K27, the Y641F mutant has poor efficiency for this first methylation step but increased efficiency in transferring the second and third methyl groups<sup>8,9</sup>. Thus the combined action of the mutant and wild-type enzyme produces the elevated levels of H3K27me3 observed in these lymphomas<sup>8,9</sup>.

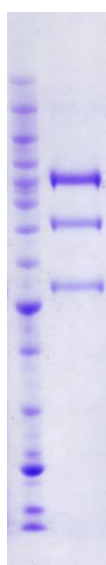
**PURITY:** >90% by SDS-PAGE.

**ASSAY CONDITIONS:** RBC's EZH2-Y641F Complex displays histone methyltransferase activity at enzyme concentrations of 15.6 nM and above, 30°C, as TCA-precipitated counts in a scintillation/filter plate assay (Multiscreen FB, Topcount) with HeLa oligo or mono/di-nucleosomes (0.05 mg/mL as [DNA]), chicken core histones (0.05 mg/mL) or recombinant histone H3.3 (1  $\mu$ M). Reaction conditions are: 50 mM Tris-HCl, pH 8.5, 50 mM NaCl, 5 mM MgCl<sub>2</sub>, 1 mM DTT, 1 mM PMSF, with substrates at concentrations indicated above and [<sup>3</sup>H]-SAM (see figure below).

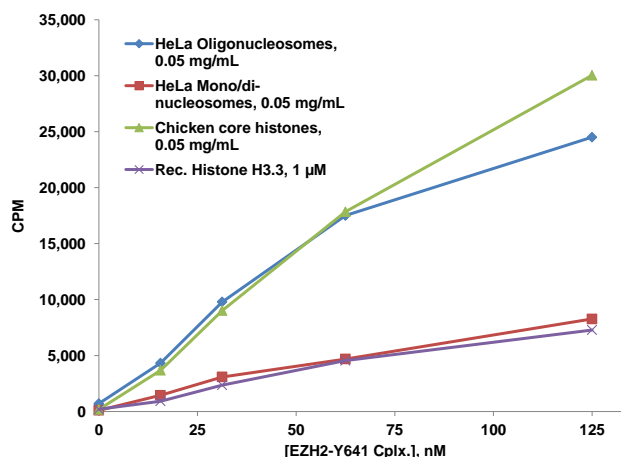
**SUPPLIED AS:** \_\_\_  $\mu$ M EZH2-Y641F Complex, as defined above, (\_\_\_  $\mu$ g/ $\mu$ l total protein) in 20 mM Tris-HCl, pH 7.9, 150 mM NaCl, 2 mM MgCl<sub>2</sub>, 2 mM DTT, 20% glycerol (w/v), 0.01% NP-40 as determined by OD<sub>280</sub>.

**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) R.D. Morin *et al. Nat. Genet.* 2010 **42** 181; 8) D.B. Yap *et al. Blood* 2011 **117** 2451; 9) C.J. Sneeringer *et al. Proc. Natl. Acad. Sci. USA* 2010 **107** 20980



**Coomassie blue stained SDS-PAGE (4-12% acrylamide) of 4  $\mu$ g of the purified EZH2-Y641F Complex.** MW markers at left, from top: 220, 160, 120, 100, 90, 80, 70, 60, 50, 40, 30, 25, 20, 15, 10 kDa. Lines at right of EZH2-Y641F Complex lane indicate positions of EZH2-Y641F-88 kDa, SUZ12-86 kDa, AEBP2-57 kDa, EED-51 kDa, RbAp48-51 kDa



**Methyltransferase Activity of the EZH2-Y641F Complex.** Methylation determined as TCA-precipitable counts in a scintillation/filter plate assay. Reactions were 60 min., 30°C with 1  $\mu$ M [<sup>3</sup>H]-SAM and the indicated protein substrates.

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