REACTION BIOLOGY

PRODUCT DATASHEET

EZH2 Complex

(Enhancer of Zeste Homolog 2 in complex with AEBP2, EED, RbAp48 & SUZ12)

CATALOG NO.: HMT-25-114 **LOT NO.:**

DESCRIPTION: Human recombinant EZH2 (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 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 expression[1-5]. EZH2, which includes a SET methyltransferase domain, is the catalytic subunit of PRC2[1,6]. 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)[6]. 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[7]). Depletion of EZH2 and/or other PRC2 components can inhibit growth or induce apoptosis in cancer cells[6, 8-10]. Consequently, EZH2 is considered a promising target for the development of anti-cancer therapies[11].

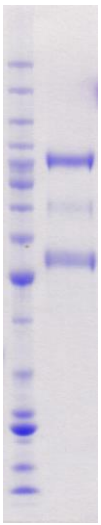
PURITY: >90% by SDS-PAGE.

ASSAY CONDITIONS: RBC's EZH2 Complex displays histone methyltransferase activity at enzyme concentrations of 13 nM and above, 30°C, with chicken core histones (0.05 mg/mL) or calf thymus histone H3 (5 µM) in the HMT HotSpot℠ Assay format or 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 [^{3}H]-SAM.

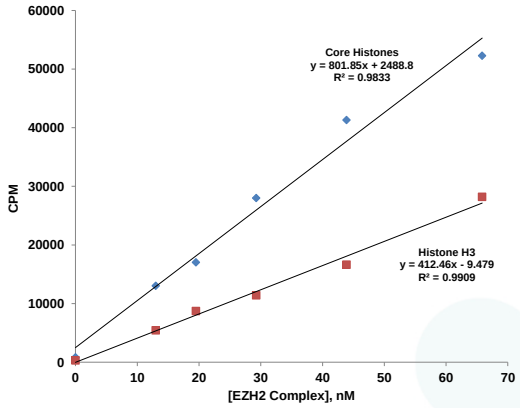
SUPPLIED AS: __ µM EZH2 Complex, as defined above, (__ µg/µl total protein) in 20 mM Tris-HCl, pH 7.9, 150 mM NaCl, 2 mM $MgCl_2$, 2 mM DTT, 20% glycerol (w/v), 0.01% NP-40 as determined by OD_{280}.

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) 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 2 µg of the purified EZH2 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, AEBP2-57 kDa, EED-51 kDa. RbAp48-51 kDa



Methyltransferase Activity of the EZH2 Complex. Methylation determined as TCA-precipitable counts in in a scintillation/filter plate assay. Reactions were 60 min., 30°C, 1 µM [^{3}H]-SAM with 5 µM histone H3 or 0.05 mg/mL core histones.

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