REACTION BIOLOGY

PRODUCT DATASHEET

G9a-GLP

CATALOG NO.: HMT-12-104 **LOT NO.:**

DESCRIPTION Equimolar mixture of human recombinant G9a (residues 786-1210; Genbank Accession # NM_006709; MW = 80 kDa) and human recombinant GLP (residues 894-1298; Genbank Accession # NM_024757; MW = 80 kDa). Both proteins expressed as an N-terminal GST-fusion protein in *E. coli.* Catalyzes the transfer of methyl groups from S-adenosyl-L-methionine (SAM) to the ε-amino function of protein L-lysine residues, especially of lysine-9 of histone H3 (H3K9)[1,2]. *In vivo,* a heterodimeric complex of the related SET-domain histone methyltransferases (HMTs) G9a and GLP is the major source of mono- and dimethylated histone H3K9 in euchromatin[3,4], marks associated with recruitment of HP1, DNA methylation and gene silencing[4-6]. A multimeric H3K9 methylation complex containing G9a/GLP along with other HMTs (SETDB1, SUV39H1) has been described[7]. The G9a-GLP association is mediated by their SET domains[4], which are present in the two constructs constituting RBC's G9a-GLP. Per mole of G9, GLP or (G9a + GLP), the mixture displays activity well in excess of G9a alone, similar to the more active GLP and greater than both the mean of G9a and GLP activities and the sum of separate G9a/GLP activities, each obtained at half the indicated enzyme concentration (see figure, below). This would suggest a significant degree of heterodimer formation occurs in the mixture.

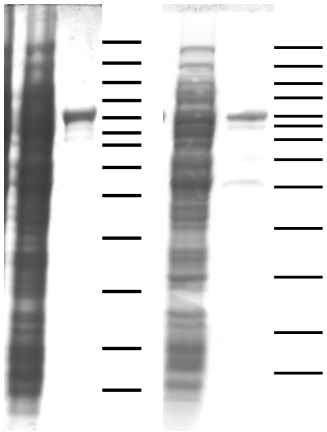
PURITY: >85% by SDS-PAGE.

ASSAY CONDITIONS: RBC's G9a-GLP displays histone methyltransferase activity at enzyme concentrations of 2 nM and above, 30°C, with calf thymus histone H3 or chicken core histones in the HMT HotSpot^{SM} Assay format. Reaction conditions are: 50 mM Tris-HCl, pH 8.5, 50 mM NaCl, 5 mM $MgCl_2$, 1 mM DTT, 1 mM PMSF, 0.05 mg/mL chicken core histones (0.05 mg/mL) or calf thymus histone H3 (5 µM), [^{3}H]-SAM.

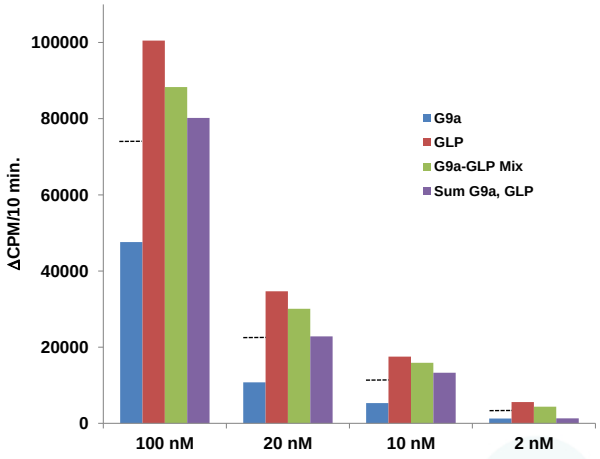
SUPPLIED AS: __ µg/µl in 25.4 mM Na_2HPO_4/NaH_2PO_4, pH 7.4, 137 mM NaCl, 2.7 mM KCl, 3 mM DTT, 30% (w/v) glycerol as determined by OD_{280}

STORAGE: -70°C. Thaw quickly and store on ice before use. The remaining, unused, undiluted enzyme should be refrozen quickly by, for example, snap freezing in a dry/ice ethanol bath or liquid nitrogen. Freezing and storage of diluted enzyme is not recommended.

REFERENCES: 1) M. Tachibana *et al. J. Biol. Chem.* 2001 **276** 25309; 2) Y. Shinkai & M. Tachibana *Genes Dev.* 2011 **25** 781; 3) M. Tachibana *et al. Genes Dev.* 2002 **16** 1779; 4) M. Tachibana *et al. Genes Dev.* 2005 **19** 815; 5) N. Feldman *et al. Nat. Cell Biol.* 2006 **8** 188; 6) M. El Gazzar *et al. J. Biol. Chem.* 2008 **283** 32198; 7) L. Fritsch *et al. Mol. Cell* 2010 **37** 46



Coomassie blue stained SDS-PAGE (4-20% acrylamide) of crude *E. coli* extracts and 2 µg of purified RBC G9a (left) and GLP (right). Lines to right indicate the positions of MW markers at, from top, 220, 160, 120, 100, 80, 70, 60, 50, 40, 30, 20, 15, 10 kDa.



Activity of G9a-GLP mixture compared to separate G9a and GLP. Reactions (10 µL) were run for 10 min., 30°C with 0.05 mg/mL core histones, 1 µM [^{3}H]-SAM. After denaturation with guanidinium HCL, histones were separated from unreacted SAM by binding to a reverse phase resin (C18) and then eluted for scintillation counting. Dashed lines mark the mean of G9a and GLP activities. "Sum G9a, GLP" values were calculated by adding the separate G9a and GLP activities obtained at half the indicated concentration (e.g. 100 nM "Sum G9a, GLP"= Activity of 50 nM G9a + Activity of 50 nM GLP).

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