PRODUCT DATASHEET

GLP (G9a-Like Protein; EHMT1 (Euchromatic Histone-lysine N-Methyltransferase 1); H3-K9-HMTase 5; KMT1D)

CATALOG NO.: HMT-11-103 **LOT NO.:**

DESCRIPTION: Human recombinant GLP (residues 874-1298; Genbank Accession # NM_024757) expressed as an N-terminal GST-fusion protein in *E. coli.* MW = 74.1 kDa. Catalyzes the transfer of methyl groups from S-adenosyl-L-methionine (SAM) to the ε-amino function of protein L-lysine residues (mono-, di- and trimethylation), especially of lysine-9 of histone H3 (H3K9)[1], but with reported activities on H3K27[6], histone H1.4K26[2], p53 K373[3] and other targets (see review[4]). GLP is a SET-domain type histone methyltransferase (HMT), which, in complex with the highly homologous G9a, is the major source of mono- and dimethylated histone H3K9 in euchromatin[5,6], marks associated with recruitment of HP1, DNA methylation and gene silencing[6-8]. A multimeric H3K9 methylation complex containing G9a/GLP along with other HMTs (SETDB1, SUV39H1) has been described[9]. GLP and G9a are overexpressed in a variety of cancers and knockdown of G9a/GLP in the MCF7 breast cancer line increases apoptosis[3]. These results, along with the fact that dimethylation at the G9a/GLP target site, p53 K373, correlates with levels of inactive p53, suggest G9a/GLP inhibition as a potential anti-cancer therapy, especially for tumors expressing wild-type p53[3].

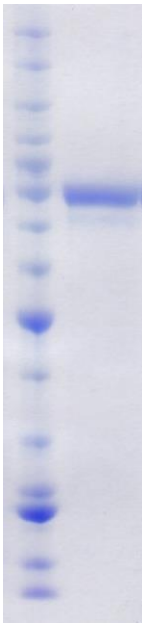
PURITY: >80% by SDS-PAGE.

ASSAY CONDITIONS: RBC's GLP displays histone methyltransferase activity at enzyme concentrations of 3.9 nM and above, 30°C, with chicken core histones or calf thymus histone H3 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.

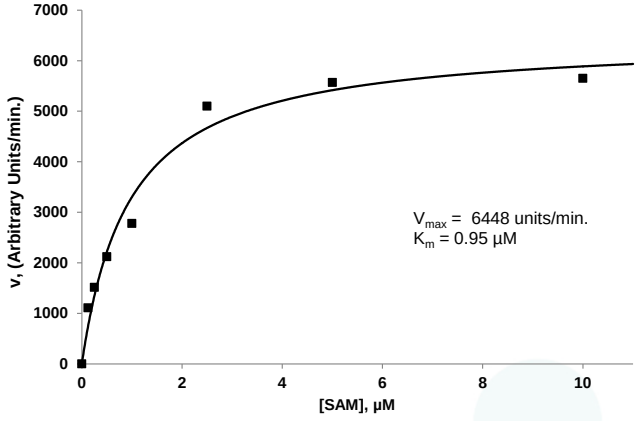
SUPPLIED AS: __ µg/µl in 25.4 mM Na_2HPO_4/NaH_2PO_4, pH 7.4, 137 mM NaCl, 2.7 mM KCl, 3 mM DTT, 30% (w/v) glycerol as determined by OD_{280}

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) H. Ogawa *et al. Science* 2002 **296** 1132; 2) T. Weiss *et al. Epigenetics Chromatin* 2010 **3** 7; 3) J. Huang *et al. J. Biol. Chem.* 2010 **285** 9636; 4) Y. Shinkai & M. Tachibana *Genes Dev.* 2011 **25** 781; 5) M. Tachibana *et al. Genes Dev.* 2002 **16** 1779; 6) M. Tachibana *et al. Genes Dev.* 2005 **19** 815; 7) N. Feldman *et al. Nat. Cell Biol.* 2006 **8** 188; 8) M. El Gazzar *et al. J. Biol. Chem.* 2008 **283** 32198; 9) L. Fritsch *et al. Mol. Cell* 2010 **37** 46



Coomassie blue stained SDS-PAGE (4-12% acrylamide) 4 µg of purified RBC GLP. MW marker (left), from top, 220, 160, 120, 100, 90, 80, 70, 60, **50**, 40, 30, 25, **20**, 15, 10 kDa.



Dependence of GLP methylation velocity on the concentration of S-adenosylmethionine (SAM). Assays were performed in the HotSpot^{SM} format with 0.05 mg/mL chicken core histones. Velocities are slopes from linear plots of 15, 30 & 45 min. time points, each derived from four determinations. Signal/Background for these time points averaged 13.2. The line, V_{max} and K_m derive from a non-linear fit to the Michaelis-Menten equation.

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