PRODUCT DATASHEET

G9a (EHMT2 (Euchromatic Histone-lysine N-Methyltransferase 2); H3-K9-HMTase 3; KMT1C)

CATALOG NO.: HMT-11-102 **LOT NO.:**

DESCRIPTION: Human recombinant G9a (residues 786-1210; Genbank Accession # NM_006709) expressed as an N-terminal GST-fusion protein in *E. coli.* MW = 80 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[1], histone H1.4K26[2], p53 K373[3] and other targets (see review[4]). G9a is a SET-domain type histone methyltransferase (HMT), which, in complex with the highly homologous GLP, 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]. G9a is 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].

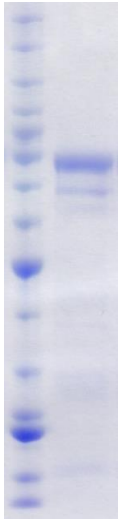
PURITY: >80% by SDS-PAGE.

ASSAY CONDITIONS: RBC's G9a displays histone methyltransferase activity at enzyme concentrations of 6.25 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.

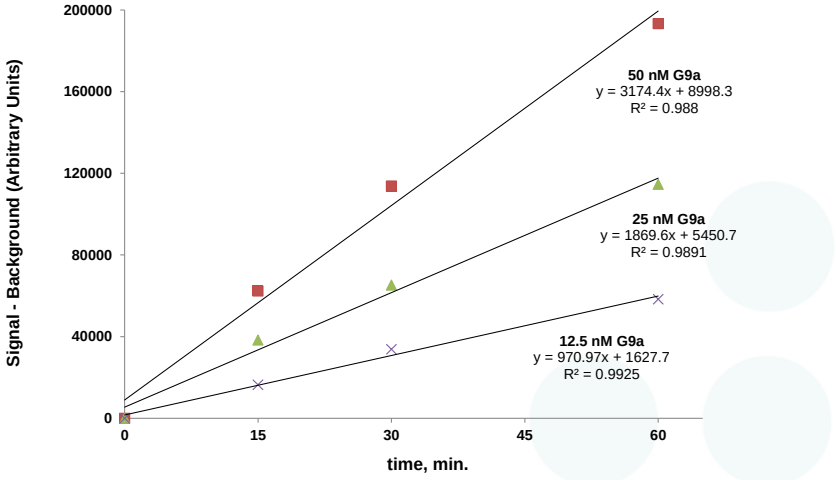
SUPPLIED AS: ___µg/µl in 25.4 mM Na_2HPO_4, pH 7.4, 4.4 mM KH_2PO_4, 137 mM NaCl, 3 mM DTT, 30% (w/v) glycerol as determined by OD_{280}

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) P. Trojer *et al. J. Biol. Chem.* 2009 **284** 8395; 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 G9a. MW marker (left), from top, 220, 160, 120, 100, 90, 80, 70, 60, **50**, 40, 30, 25, **20**, 15, 10 kDa.



Time courses of G9a methyltransferase reactions in the HotSpot^{SM} assay format. G9a, at the indicated concentrations, was assayed with 0.05 mg/mL chicken core histones and 1 µM [^{3}H]-SAM. Signal/Background ranged from 4.3 (12.5 nM G9a, 15 min.) to 51.2 (50 nM G9a, 60 min.).

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