# REACTION BIOLOGY

# PRODUCT DATASHEET

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

**CATALOG NO.:** HMT-11-245 **LOT NO.:**

**DESCRIPTION:** Human recombinant G9a (residues 913-1193; Genbank Accession #NM_006709.3, MW = 34.6 kDa) expressed with an N-terminal His-tag 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 (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].

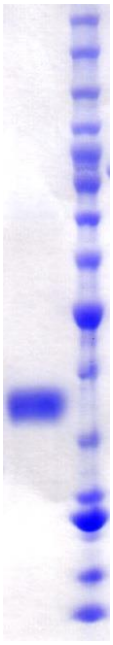
**PURITY:** >90% by SDS-PAGE.

**ASSAY CONDITIONS:** RBC's G9a (aa913-1193) displays histone methyltransferase activity at enzyme concentrations of 0.4 nM and above, 25°C, with biotinylated H3 (1-21 ) peptide in the SPA assay format, measured in the TopCount$^{SM}$ Reaction conditions are: 20 mM Tris-HCl, pH 8.0; 0.01% Triton X100; 2 µM b-H3(1-21), 9 µM SAM, 1 µM [$^3$H]-SAM.

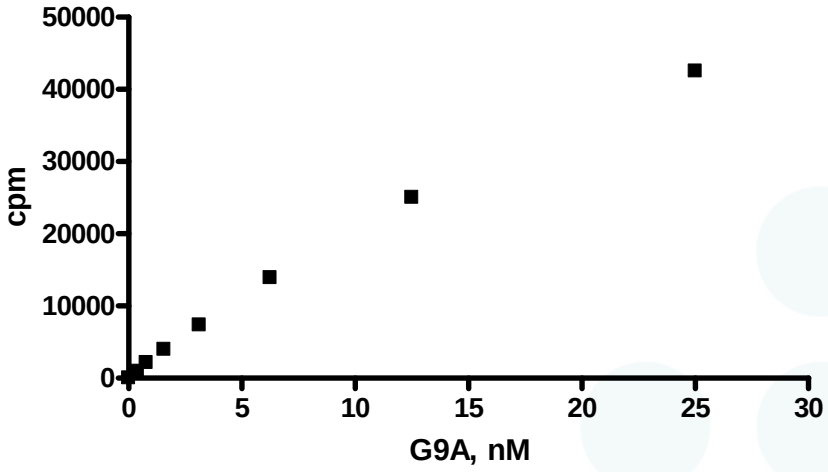
**SUPPLIED AS:** ___µg/µl in 50 mM Tris-HCl pH 7.5, 500 mM NaCl, 1 mM TCEP, 10% (w/v) glycerol

**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 (12% acrylamide) of 2µg of RBC G9a (His)**
MW markers (right) are, from top, 220, 160, 120, 100, 90, 80, 70, 60, **50**, 40,




**Activity of G9A (aa 913-1193) methyltransferase in SPA assay format**. Variable concentrations of G9A were incubated with 2 µM biotinylated H3(1-21) peptide and 10 µM SAM (10% $^3$H-SAM) for 45 minutes. Reactions were quenched, transferred into streptavidin SPA plates (Perkin Elmer) containing 20 mM Tris pH 8.0 buffer and incubated for 1h at RT. SPA plate was read using Top Count scintillation counter (Perkin Elmer).



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