

**PRDM9**

(PR Domain Zinc Finger Protein 9; Meisetz)

**CATALOG NO.:** HMT-21-152

**LOT NO.:**

**DESCRIPTION:** Human recombinant PRDM9, (residues 2-414); Genbank Accession # NM\_020227; MW = 76.3 kDa) expressed in *Sf9* insect cells with an N-terminal GST-tag. Catalyzes the transfer of methyl groups from S-adenosyl-L-methionine (SAM) to the ε-amino function of protein L-lysine residues, specifically histone H3 lysine-4 (H3K4)<sup>1</sup>. Expressed only in meiotic male and female germ cells, PRDM9 is required for fully-functional pairing of homologous chromosomes, double strand break repair, meiotic progression and fertility<sup>1</sup>. PRDM9 binds, via its C-terminal array of zinc fingers (ZnFs), to sequence motifs associated with “hotspots” for homologous recombination (crossovers)<sup>2-6</sup>. Such hotspots are enriched for trimethylated histone H3 lysine-4 (H3K4me3)<sup>3,7,8</sup>, presumably the result of PRDM9’s H3K4 methyltransferase activity<sup>1,8</sup>, and part of PRDM9’s role may be to direct meiotic recombination events away from non-PRDM9 H3K4me3 marks, such as those in promoters<sup>9</sup>. Consistent with its requirement in meiotic recombination and the rapid evolution of its ZnFs<sup>5,10</sup>, there is evidence to suggest roles for PRDM9 in evolution and speciation<sup>5,10-12</sup> (see also review<sup>13</sup> and references therein), genomic instability<sup>4,14</sup> and the risk of childhood leukemia<sup>15</sup>. PRDM9’s histone methyltransferase activity has received relatively little study, although results with the *E. coli*-expressed mouse enzyme imply that it is an H3K4 trimethylase that only catalyzes the H3K4me2 to H3K4me3 transition<sup>1</sup>. However, since RBC’s insect cell-expressed human enzyme is most active with, among the substrates tested, the presumably unmethylated *E. coli*-expressed histone H3.3 (RBC Cat. # HMT-11-134), this would suggest that it is capable of other methylations as well (see figure below).

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

**ASSAY CONDITIONS:** RBC’s PRDM9 displays histone methyltransferase activity with recombinant histone H3.3 (RBC Cat. # HMT-11-134) and, to a lesser extent, with chicken core histones, in a radiolabeled scintillation/filter plate assay as TCA-precipitated counts (Multiscreen FB, Topcount). Reaction conditions are: 50 mM Tris-HCl, pH 8.5, 50 mM NaCl, 5 mM MgCl<sub>2</sub>, 1 mM DTT, 1 mM PMSF, substrates at concentrations indicated (see Figure below).

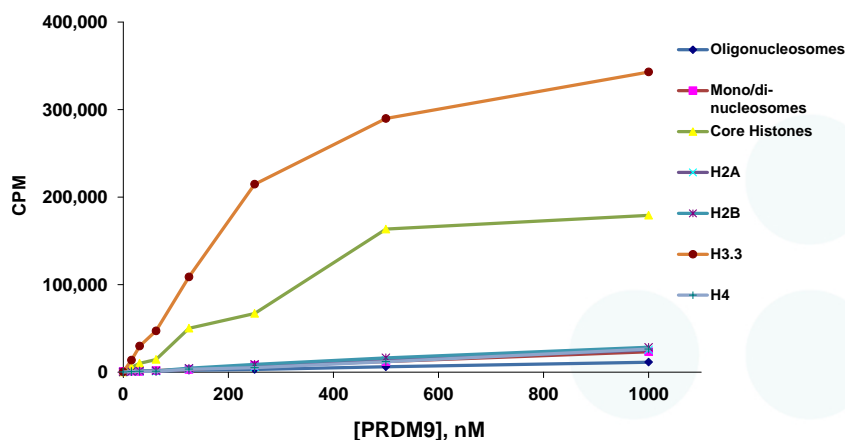
**SUPPLIED AS:** \_\_\_ μg/μl total protein in 50 mM Tris/HCl pH 7.5, 500 mM NaCl, 1 mM TCEP, 10% glycerol (v/v) as determined by OD<sub>280</sub>.

**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) K. Hayashi *et al. Nature* 2005 **438** 374; 2) E.D. Parvanov *et al. Science* 2009 **327** 835; 3) C. Grey *et al. PLOS Biol.* 2009 **7** e35; 4) I.L. Berg *et al. Nature Genet.* 2010 **42** 859; 5) S. Myers *et al. Science* 2010 **327** 876; 6) F. Baudat *et al. Science* 2010 **327** 836; 7) J. Buard *et al. EMBO J.* 2009 **28** 2616; 8) C. Grey *et al. PLOS Biol.* 2011 **9** e1001176; 9) K. Brick *et al. Nature* 2012 **485** 642; 10) P.L. Oliver *et al. PLOS Genet.* 2009 **5** e1000753; 11) O. Mihola *et al. Science* 2009 **323** 373; 12) J.H. Thomas *et al. PLOS One* 2009 **4** e8505; 13) K. Nowick *et al. Trends Genet.* 2013 **29** 130; 14) A.J. Jeffreys *et al. Proc. Natl. Acad. Sci. USA* 2013 **110** 600; 15) J. Hussin *et al. Genome Res.* 2013 **23** 419



Coomassie blue stained SDS-PAGE (4-12% acrylamide) of 4 μg of purified PRDM9. MW markers at left, are from top: 220, 160, 120, 100, 90, 80, 70, 60, 50, 40, 30, 25, 20, 15, & 10 kDa.



**Methylation Activity of PRDM9.** Assays were performed with a scintillation/filter plate assay. Incubations were 60 min., 30°C with 1 μM [<sup>3</sup>H]-SAM plus HeLa Mono/di- or Oligonucleosomes (0.05 mg/mL as [DNA]) or chicken core histones (0.05 mg/mL) or 1 μM histone H3.3 or 5 μM histones H2A, H2B or H4.

This product is not intended for therapeutic or diagnostic use in animals or in humans.