PRODUCT DATA SHEET

Recombinant Tetranucleosomes (human, Histone H3.3-containing)

CATALOG NO.: HMT-15-369 **LOT NO.:**

DESCRIPTION: Tetranucleosomes assembled from full-length recombinant human histones (H2A, residues 1-130; Genbank Accession # NM_021052, MW = 14.1 kDa; H2B, residues 1-126; Genbank Accession # NM_080593, MW = 13.9 kDa; H3.3, residues 1-136; Genbank Accession # NM_002107, MW = 15.3 kDa; H4, residues 2-103; Genbank Accession # NM_003538, MW = 11.2 kDa) Histone octamers are assembled into tetranucleosomes by standard methods[1-3] with an 844 bp DNA comprising four repeats of a strong nucleosome positioning sequence[4]. Each tetranucleosome unit will thus contain 8 copies each of the four histones, plus the 844 bp DNA, for a total MW of 985 kDa.

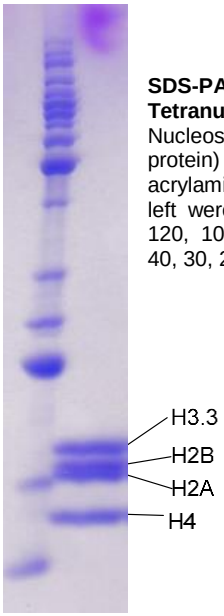
PURITY: >90% by SDS-PAGE, agarose gel electrophoresis.

APPLICATIONS: Useful for the assay of various histone methyltransferases (e.g. MLL1 Complex, NSD1 and Dot1L) either by methods employing radiolabeling with [^{3}H]-*S*-adenosylmethionine (SAM) (e.g. gel electrophoresis/autoradiography or filterplate/scintillation counting; see Figure) or, especially, by methods involving detection with antibodies specific for particular methylation sites and states (e.g. Anti-histone H3K36me2). The advantage of *E. coli*-expressed recombinant nucleosomes for the latter method is due to the negligible background level of methylation present on histones expressed in *E. coli.*

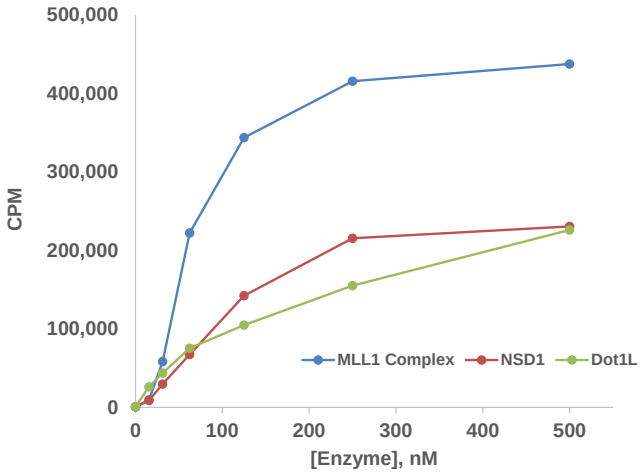
SUPPLIED AS: __ µg/µl (as [DNA]) in 20 mM HEPES pH 7.5, 1 mM EDTA, 0.5 mM PMSF, 1 mM ß-mercaptoethanol, 20% glycerol (w/v). **NOTE:** Each vial contains 50 µg nucleosomal **DNA**, determined by A_{260nm}. Given the assembly ratio of 211 bp/nucleosome, the total weight, DNA + protein, is 89.7 µg. Divide the DNA concentration (µg/µL) by 137,150 (µg/µmol), the MW of 211 bp DNA, to obtain the molarity of single nucleosomal units (histone octamer + 211 bp DNA). Multiply this molarity by 2 to obtain the molarity of any of the 4 core histones (H3, H4, H2A, H2B).

STORAGE: -70°C. Thaw quickly and store on ice before use. The remaining, unused, undiluted portion 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 solutions are not recommended.

REFERENCES: 1) K. Luger *et al. Methods Enzymol.* 1999 **304** 3; 2) Y. Tanaka *et al. Methods* 2004 **33** 3; 3) P. Dyer *et al. Methods Enzymol.* 2004 **375** 23; 4) P.T. Lowary & J. Widom *J. Mol. Biol.* 1998 **276** 19



SDS-PAGE of Recombinant Tetranucleosomes. Nucleosomes (~3 µg histone protein) were run on a 16% acrylamide gel. MW markers at left were, from top: 220, 160, 120, 100, 90, 80, 70, 60, 50, 40, 30, 25, 20, 15, 10 kDa.



Assay of MLL1 Complex, NSD1 and Dot1L Methylation Activities with Recombinant Tetraonucleosomes. Assays (25 µL) were performed with a scintillation/filter plate assay. Incubations were 60 min., 30°C with indicated concentrations of MLL1 Complex (RBC Cat. # HMT-15-105); NSD2 (RBC Cat. # HMT-21-122) or Dot1L (RBC Cat. # HMT-11-101) plus HeLa Oligonucleosomes (0.05 mg/mL as [DNA]) and 1 µM [^{3}H]-SAM.

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

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