

Nucleosomes (HeLa Oligo)

CATALOG NO.: HMT-35-130

LOT NO.:

DESCRIPTION: Oligonucleosomes purified from HeLa cells (primarily oligomers of 3-6 units, 600-1200 bp DNA), by a modification of the method of Schnitzler¹. These are H1-depleted core nucleosomes comprising histone octamers (two copies each of histones H3, H4, H2A, H2B), each wrapped with ~146 bp of DNA with ~50 additional bp of internucleosomal DNA.

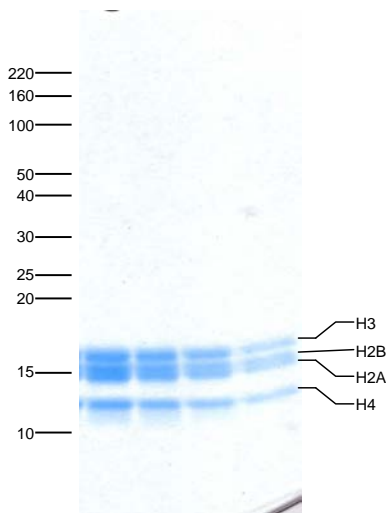
PURITY: >90% by SDS-PAGE, agarose gel electrophoresis.

APPLICATIONS: Useful for the assay of various histone methyltransferases (e.g. MLL1 Complex, MLL2 Complex, MLL4 Complex, NSD2 and Dot1L) by methods employing radiolabeling with [³H]-S-adenosylmethionine (SAM) (e.g. gel electrophoresis/autoradiography or filterplate/scintillation counting). Reaction conditions: 50 mM Tris-HCl, pH 8.5, 50 mM NaCl, 5 mM MgCl₂, 1 mM DTT, 1 mM PMSF, 0.05 mg/mL Nucleosomes (as [DNA]), 1 μM [³H]-SAM.

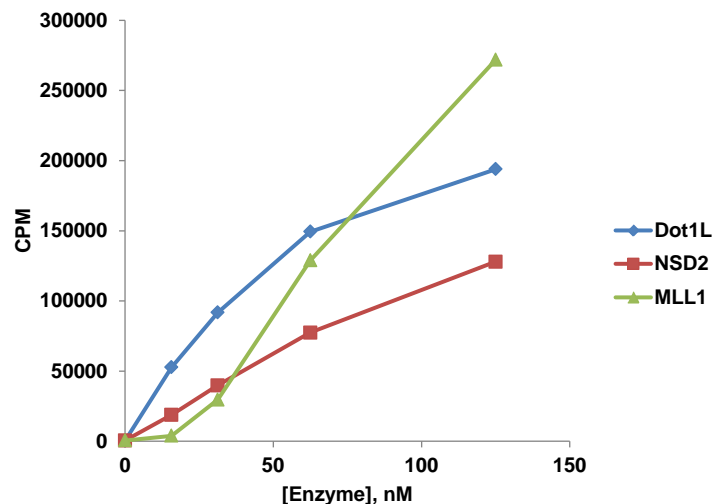
SUPPLIED AS: ___ μg/μl (as [DNA]) in 20 mM HEPES pH 7.5, 1 mM EDTA, 0.5 mM PMSF, 1 mM β-mercaptoethanol, 20% glycerol (w/v). **NOTE:** Each vial contains 50 μg nucleosomal DNA, determined by A_{260nm}. Assuming ~200 bp/nucleosome, the total weight, DNA + protein, is 91 μg. Divide the DNA concentration (μg/μL) by 130,000 (μg/μmol), the MW of ~200 bp DNA, to obtain the molarity of nucleosomal units (histone octamer + 200 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 is not recommended.

REFERENCE: 1) G. Schnitzler *Current Protocols in Molecular Biology* 2000 21.5.1-21.5.12



SDS-PAGE of HeLa Oligonucleosomes. Varying amounts of HeLa Oligonucleosomes were run on an 18% acrylamide denaturing gel (from left: 1.9, 1.4, 1.0, 0.6 μg as DNA). Bars and numbers to left indicate the positions of molecular weight markers in kDa.



Assay of MLL1 Complex, NSD2 and Dot1L Methylation Activities with HeLa Oligonucleosomes. 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 [³H]-SAM.

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