

Nucleosomes (Chicken Mono/di)

CATALOG NO.: HMT-35-179

LOT NO.:

DESCRIPTION: Mono/di-nucleosomes purified from chicken blood cells by a modification of the method of Schnitzler¹. These are H5-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: >95% by SDS-PAGE, agarose gel electrophoresis.

APPLICATIONS: Useful for the assay of various histone methyltransferases (e.g. MLL1 Complex, MLL2 Complex, MLL4 Complex, NSD2, NSD3 and Dot1L) by methods employing radiolabeling with [³H]-S-adenosylmethionine (SAM) (e.g. gel electrophoresis/autoradiography or filterplate/scintillation counting). Standard HMT 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. (See NSD3 assay buffer in figure legend, below.)

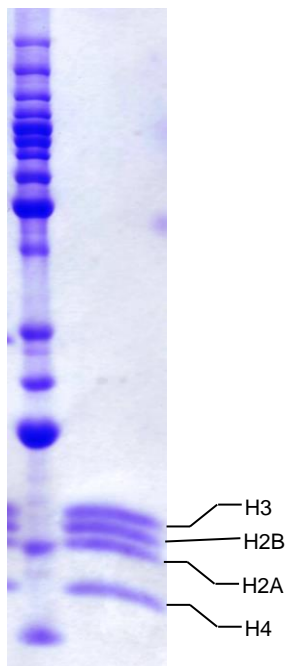
SUPPLIED AS: _ μg/μl (as [DNA]) in 20 mM HEPES pH 7.5, 1 mM EDTA, 0.5 mM PMSF, 1 mM β-mercaptoethanol, 20% glycerol (w/v). **NOTE:** Each vial contains 50 μg nucleosomal DNA, determined by A_{260nm}. Assuming ~200 bp/nucleosome, the total weight, DNA + protein, is 91 μg. Divide the DNA concentration (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.

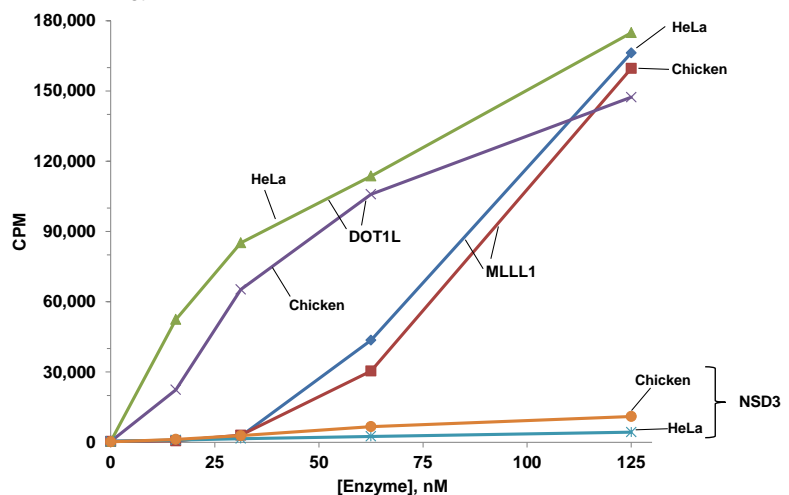
REFERENCE: 1) G. Schnitzler *Current Protocols in Molecular Biology* 2000 21.5.1-21.5.12

SDS-PAGE of Chicken Mono/di- nucleosomes.

A 16% acrylamide gel was loaded with purified chicken mono/di-nucleosomes (2 μg as DNA, ~1.6 μg protein). MW markers at left are from the top: 220, 160, 120, 100, 90, 80, 70, 60, 50, 40, 30, 25, 20, 15 & 10 kDa.



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



Most Histone Methyltransferases Have Equal or Somewhat Greater Activity with Chicken Relative to HeLa Nucleosomes. 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); NSD3 (RBC Cat. # HMT-11-132) or Dot1L (RBC Cat. # HMT-11-101) plus Chicken or HeLa Mono/di-nucleosomes (0.05 mg/mL as [DNA]) and 1 μM [³H]-SAM. Assay buffer was as above except for NSD3, for which the buffer was 40 mM Tris/HCl pH 8.8, 4 mM TCEP and 0.01% Triton X-100.