

## Nucleosomes (HeLa Mono/Di)

**CATALOG NO.:** HMT-35-123

**LOT NO.:**

**DESCRIPTION:** Mono/Di-Nucleosomes purified from HeLa cells, by a modification of the method of Schnitzler<sup>1</sup>. 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 and NSD2) by methods employing radiolabeling with [<sup>3</sup>H]-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 MgCl<sub>2</sub>, 1 mM DTT, 1 mM PMSF, 0.05 mg/mL Nucleosomes (as [DNA]), 1 μM [<sup>3</sup>H]-SAM.

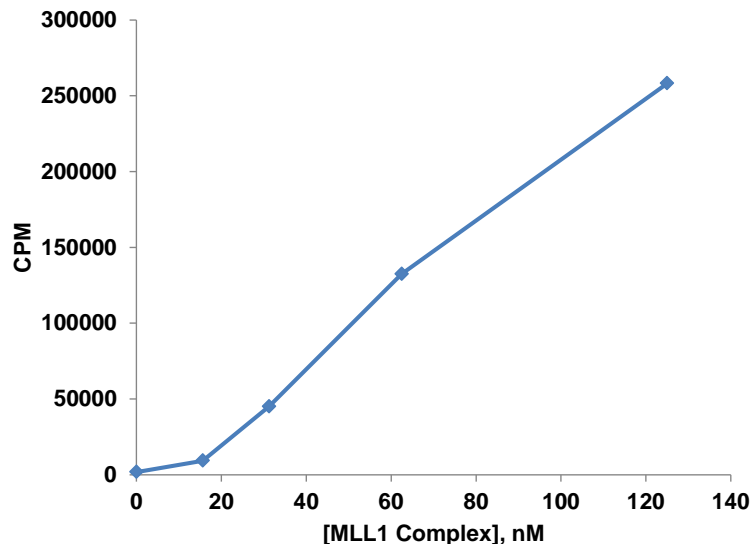
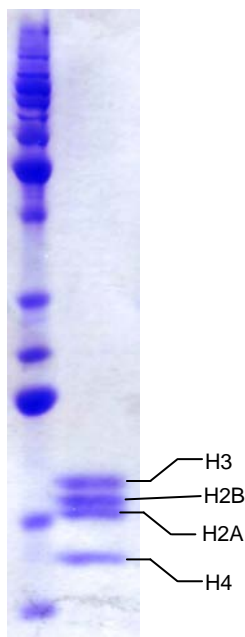
**SUPPLIED AS:** \_\_ μg/μl (as [DNA]) in 20 mM HEPES pH 7.5, 1 mM EDTA, 0.5 mM PMSF, 1 mM β-mercaptoethanol, 20% glycerol (w/v). **NOTE:** Each vial contains 50 μg nucleosomal **DNA**, determined by A<sub>260nm</sub>. Assuming ~200 bp/nucleosome, the total weight, DNA + protein, is 91 μg. Divide the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

**Coomassie Blue-Stained SDS-PAGE of HeLa Mono/di-nucleosomes.**

A 16% acrylamide gel was loaded with purified HeLa 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.



**Assay of MLL1 Complex Methylation Activity with HeLa Mono/Di-nucleosomes.** Assays were performed with a scintillation/filter plate assay. Incubations were 60 min., 30°C with MLL1 Complex (RBC Cat. # HMT-15-105); 0.05 mg/mL Nucleosomes as [DNA] and 1 μM [<sup>3</sup>H]-SAM.

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