REACTION BIOLOGY

PRODUCT DATASHEET

Nucleosomes (HeLa Oligo, Biotinylated)

CATALOG NO.: HMT-35-160 **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 Schnitzler[1]. 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. The purified oligonucleosomes are then biotinylated with an amine-reactive reagent, before dialysis into the final storage buffer. The biotinylation conditions have been optimized to achieve the highest level of modification possible while still retaining most or all of the activity normally observed with a panel of three methyltransferases (Dot1L, NSD2 and MLL1 Complex) when assayed with unmodified nucleosomes (see Figures below).

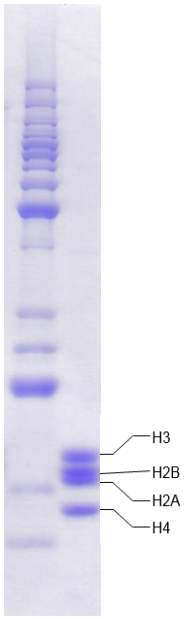
PURITY: >80% by SDS-PAGE, agarose gel electrophoresis.

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 [^{3}H]-*S*-adenosylmethionine (SAM) and scintillation proximity (e.g. streptavidin-coated Flashplates™) or by methods employing a combination of site-specific anti-methyllysine antibodies and streptavidin-conjugated reagents (e.g. AlphaLISA™). Reaction conditions: 50 mM Tris-HCl, pH 8.5, 50 mM NaCl, 5 mM $MgCl_2$, 1 mM DTT, 1 mM PMSF, 0.05 mg/mL Nucleosomes (as [DNA]), 1 µM unlabeled or [^{3}H]-SAM.

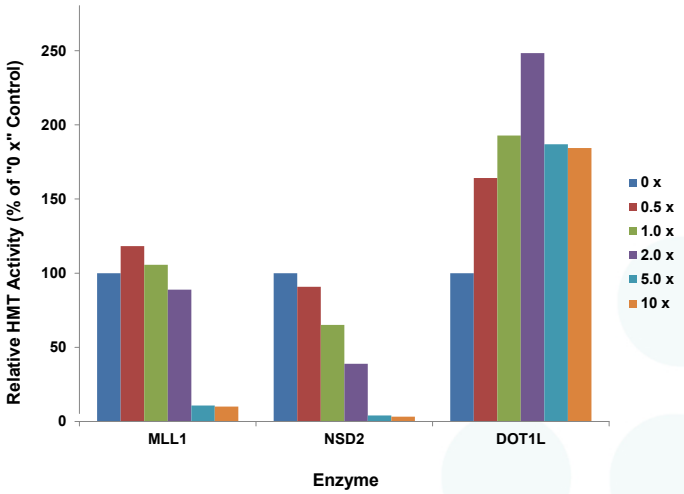
SUPPLIED AS: __ µg/µl (as [DNA]) in 20 mM HEPES pH 7.5, 1 mM EDTA, 0.5 mM PMSF, 1 mM ß-mercaptoethanol, 20% glycerol (w/v). **NOTE:** Each vial contains 50 µg nucleosomal **DNA**, determined by A_{260nm}. Assuming ~200 bp/nucleosome, the total weight, DNA + protein, is 91 µg. Divide the DNA concentration (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 (16% acrylamide) of 2 µg DNA, 0.18µg histones of RBC Nucleosomes (HeLa Oligo, Biotinylated). MW markers (left) are, from top, 220, 160, 120, 100, 90, 80, 70, 60, **50**, 40, 30, 25, **20**, 15, 10 kDa.



Relative Activities of MLL1 Complex, NSD2 and Dot1L with Unmodified and Biotinylated HeLa Oligonucleosomes. Assays (25 µL) were performed with 62.5 nM MLL1 Complex, 15.6 nM NSD2 or 31.3 nM Dot1L in a scintillation/filter plate assay (conditions given above). Activity with unmodified nucleosomes ("0 x", dark blue) is set at 100. "0.5 x", "1.0 x","2.0 x" etc. indicate relative concentrations of biotinylation reagent used to treat the nucleosomes for the indicated assay. Relative activity with RBC's product, **"Nucleosomes (HeLa Oligo, Biotinylated)"** (Cat. #HMT-35-160) is indicated by the "1.0 x" (green) columns.

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