

Nucleosomes (Chicken Oligo/Poly H5 Enriched)

CATALOG NO.: HMT-35-179

LOT NO.:

DESCRIPTION: Oligonucleosomes purified from chicken blood cells (primarily oligomers of 3-6 units, 600-1200 bp DNA), by a modification of the method of Schnitzler¹. These are H5 containing 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, 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.

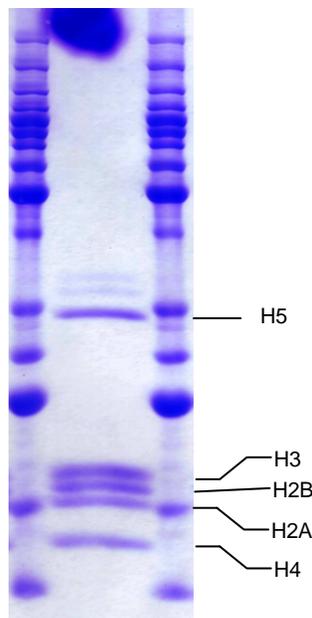
REFERENCE: 1) G. Schnitzler *Current Protocols in Molecular Biology* 2000 21.5.1-21.5.12

SDS-PAGE of Chicken Oligonucleosomes.

A 16% acrylamide gel was loaded with purified chicken oligonucleosomes (2 μg as DNA, ~1.6 μg protein).

Lane 2 contains H5 enriched nucleosomes, (RBC Product HMT-35-179)

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.