

Histone H3.3

CATALOG NO.: HMT-11-134

LOT NO.:

DESCRIPTION: Full-length, untagged human recombinant histone H3.3 (residues 2-136; Genbank Accession # NM_002107; MW = 15.3 kDa) expressed in *E. coli*. Histone H3.3 is incorporated into chromatin independently of replication and is enriched in areas of active gene transcription¹⁻⁶. RBC's Histone is useful for the assay of various histone methyltransferases (e.g. MLL2 Complex, SUV39H1, G9a, GLP) either by radiolabeling with [³H]-S-adenosylmethionine (e.g. gel electrophoresis/autoradiography or filterplate/scintillation counting) or by methods employing site/modification-specific antibodies (e.g. anti-H3K4me2).

PURITY: >90% by SDS-PAGE.

ASSAY CONDITIONS: Methyltransferase assays (see figure, below) were performed with histone H3.3. and [³H]-SAM as substrates. Activity was determined as TCA-precipitated counts in a scintillation/filter plate assay (Multiscreen FB, Topcount). Reaction conditions: 50 mM Tris-HCl, pH 8.5, 50 mM NaCl, 5 mM MgCl₂, 1 mM DTT, 1 mM PMSF, 30°C, 60 min.

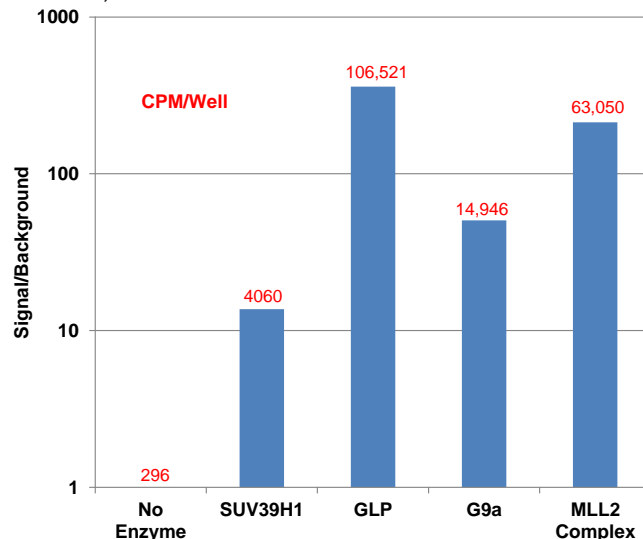
SUPPLIED AS: ___ µg/µl total protein in in 50 mM HEPES/NaOH pH 7.1, 10 mM KCl, 10% glycerol (v/v) as determined by OD₂₈₀

STORAGE: -70°C. Thaw quickly and store on ice before use. The remaining, unused, undiluted protein 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 enzyme is not recommended.

REFERENCES: 1) K. Ahmad & S. Henikoff *Mol. Cell* 2002 **9** 1191; 2) E. McKittrick *et al. Proc.Natl Acad. Sci.USA.* 2004 **101** 1525; 3) H. Tagami *et al. Cell* 2004 **116** 51; 4) C.-M. Chow *et al. EMBO Rep.* 2005 **6** 354; 5) L. Daury *et al. EMBO Rep.* 2006 **7** 66; 6) E. Delbarre *et al. Mol. Biol. Cell* 2010 **21** 1872;



Coomassie blue stained SDS-PAGE (4-12% acrylamide) of 4 µg of purified Histone H3.3. MW markers at left, from top: 220, 160, 120, 100, 90, 80, 70, 60, 50, 40, 30, 25, 20, 15, 10 kDa.



Assay of Methyltransferases with Histone H3.3. The indicated enzymes were assayed at 15.6 nM, 60 min., 30°C, with 1 µM [³H]-SAM and 1 µM histone H3.3 as substrates, in 25 µL reactions. Columns represent the signal to background (no enzyme) ratio, plotted on a log scale. The red numbers are the TCA-precipitable counts per min. determined in a scintillation/filter plate assay. Enzymes used were RBC cat. #'s HMT-11-111 (SUV39H1), HMT-11-103 (GLP), HMT-11-102 (G9a), HMT-15-106 (MLL2 Complex).

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