PRODUCT DATA SHEET

Histone H1.0 (H1(0), H1')

CATALOG NO.: HMT-11-180 **LOT NO.:**

DESCRIPTION: Full-length, untagged human recombinant histone H1.0 (H1(0), H1'; residues 2-195; Genbank Accession # NM_005318; MW = 20.9 kDa) expressed in *E. coli*. H1 histones function as 'linkers' that bind to internucleosomal DNA (~1:1 with the histone octamer of the nucleosome core), converting nucleosomes to "chromatosomes" and promoting the formation of more compact, higher-order chromatin structure[1,2] (see also review[3]). Histone H1.0, the H1 variant most homologous to the H5 histone of avian erythrocytes, is, along with histones H1.1-H1.5, one of six H1 variants found in terminally differentiated cells[5]. Recent work indicates that aside from playing a role in chromatin structure and function, histone H1.0 interacts with a wide array of nucleolar proteins, with specific interactions implying a role in rRNA splicing and ribosome biogenesis[6]. In general, phosphorylation is the predominant H1 post-translational modification[7], notably associated with cell cycle progression[8], but other modifications, including lysine acetylation[9]/methylation[10] and ADP-ribosylation[10], are also reported (see also review[3]). Crosstalk occurs between H1 modifications and those on other histones[10] and H1 variants can affect particular histone modifications, e.g. H1.3 (mouse H1d) inhibition of SET7/9 H3K4 methylation[11] (see also Figure below), consistent with a role for H1 in transcriptional regulation. Indeed, gene-specific transcriptional regulatory effects of H1 variants have been reported[12], but these remain less well studied than H1's role in chromatin structure.

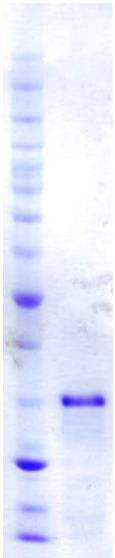
PURITY: >90% by SDS-PAGE.

ASSAY CONDITIONS: Kinase assays (see figure below) were performed in the HotSpot^{SM} assay system, at room temperature, with 2 hr. reactions and 20 µM histone H1 and 10 µM [γ-^{33}P]ATP as substrates. For methyltransferase assays (see figure, below) histone H1 was added in molar excess to H1-depleted HeLa oligonucleosomes (Cat. # HMT-35-130) and oligonucleosomes with incorporated histone H1, were then isolated by glycerol gradient centrifugation. 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 $MgCl_2$, 1 mM DTT, 1 mM PMSF, 30°C, 60 min. with 1 µM [^{3}H]. $MgCl_2$ was omitted from the buffer, where indicated (see figure).

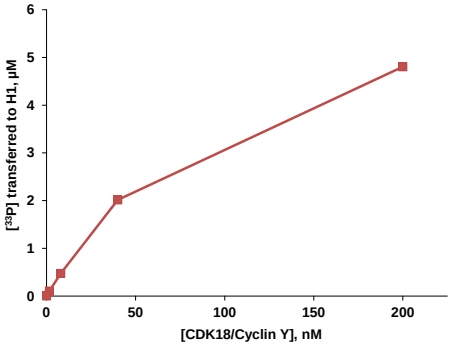
SUPPLIED AS: ___ µg/µl total protein in 50 mM Tris/HCl pH 7.5, 750 mM NaCl, 1 mM TCEP, 10% glycerol (v/v) as determined by OD_{280}

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 protein is not recommended.

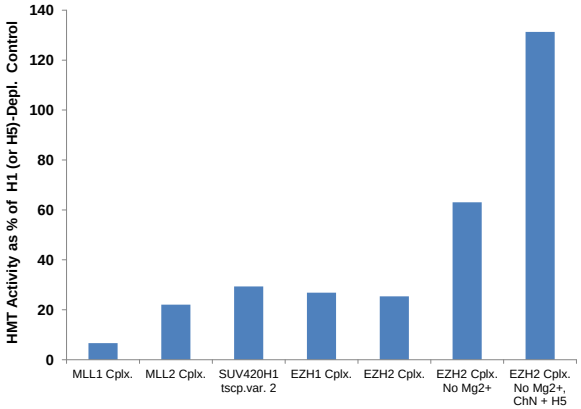
REFERENCES: 1) X. Lu & J.C. Hansen *J. Biol. Chem.* 2004 **279** 8701; 2) T.L. Caterino *Mol. Cell. Biol.* 2011 **31** 2341; 3) S.W. Harshman *et al. Nucl. Acids Res.* 2013 **41** 9593; 4) E. Schulze & B. Schulze *J. Mol. Evol.* 1995 **41** 833; 5) J.P.H. Th'ng *et al. J. Biol. Chem.* 2005 **280** 27809; 6) A.A. Kalashnikova *et al. Nucl Acids Res.* 2013 **41** 4026; 7) B. Sarg *et al. Mol. Cell Proteomics* 2013 **12** 2640; 8) H. Talasz *et al. Biochemistry* 1996 **35** 1761; 9) K.K. Kamieniarz *et al. Genes Dev.* 2012 **26** 797; 10) I. Kassner *et al. Epigenetics & Chromatin* 2013 **6** 1; 11) S.-M. Yang *et al. Proc. Natl. Acad. Sci. USA* 2013 **110** 1708; 12) Y. Zhang *et al. PLoS One* 2012 **7** e38829



Coomassie blue stained SDS-PAGE (4-12% acrylamide) of 2 µg of purified Histone H1. MW markers at left, from top: 220, 160, 120, 100, 90, 80, 70, 60, **50**, 40, 30, 25, **20**, 15, 10 kDa. Note that the histone H1 migrates to an anomalously high position in this gel system, approximately the same as the 30 kDa marker.



Histone H1 Can Serve as a Kinase Substrate. The kinase CDK18/Cyclin Y was titrated in the HotSpot^{SM} assay system with 20 µM histone H1 and 10 µM [γ-^{33}P]ATP as substrates. See assay conditions above.



Incorporation of Histone H1.0 into Oligonucleosomes Decreases the Activity of Various Methyltransferases. Methylation activity of the indicated enzyme on HeLa oligonucleosomes with incorporated recombinant H1.0 is shown as a percentage of the activity on H1-depleted HeLa Oligonucleosomes. The inhibitory effect on EZH2 activity is somewhat diminished in the absence of $MgCl_2$ in the assay buffer. In contrast to the HeLa nucleosome result, EZH2 has modestly enhanced activity on chicken erythrocyte oligonucleosomes containing the H1 ortholog histone H5 (ChN + H5) relative to H5-depleted oligonucleosomes. Percent activities were calculated from the slopes of the linear portions of enzyme titration plots with nucleosomes +/– H1 or H5.

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

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