REACTION BIOLOGY CORP.

PRODUCT DATA SHEET

Histone H2B

CATALOG NO.: HMT-11-147 **LOT NO.:**

DESCRIPTION: Full-length, untagged human recombinant histone H2B (residues 1-126; Genbank Accession # NM_080593; MW = 13.9 kDa) expressed in *E. coli*. One of the four, core nucleosomal histones, H2B forms dimers with histone H2A, two of which associate with the H3-H4 tetramer $(H3\text{-}H4)_2$, forming the core octamer of the nucleosome (see review[1]). Histone H2B is subject to several regulatory post-translational modifications including monoubiquitination[2], phosphorylation[3], acetylation[4], lysine methylation[4] and crotonylation[5]. Monoubiquitination of H2B lysine-120 (H2BK120ub) stimulates H3K4 methylation in chromatin[6], an effect which recent evidence suggests is mediated in trans via ubiquitin binding by Ash2L, a component of the human MLL/SET1 family methyltransferase complexes[7]. Monoubiquitination at both H2BK34[8] and H2BK120[9] has been shown to enhance H3K79 methylation by Dot1L. RBC's Histone useful for the assay of histone acetyltransferases (HATs; e.g. p300, CBP; see Figure, below) either by radiolabeling with [^{3}H]-*S*-adenosylmethionine (e.g. gel electrophoresis/autoradiography or filterplate/scintillation counting) or by non-radiolabeled methods (e.g. detection with site-specific anti-acetyllysine antibodies).

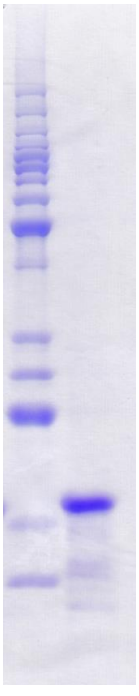
PURITY: >90% by SDS-PAGE.

ASSAY CONDITIONS: Histone acetyltransferase assays (see figure, below) were performed with histone H2B and [^{3}H]-Acetyl-CoA 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 8.0, 50 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 0.1 mM PMSF, 30°C, 60 min.

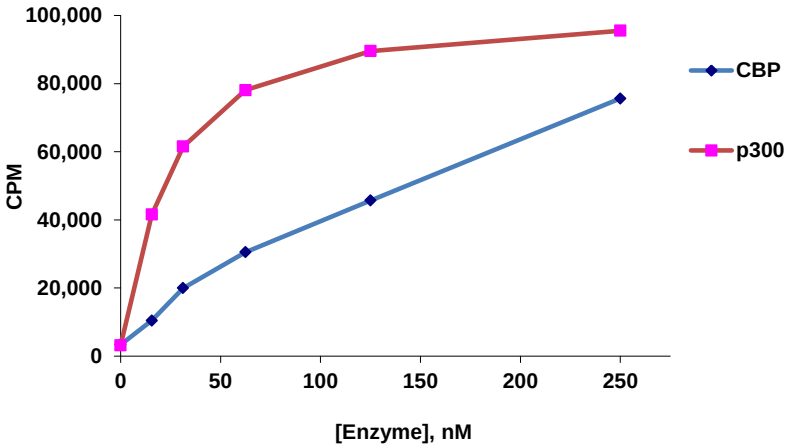
SUPPLIED AS: ___ µg/µl total protein in in 20 mM sodium phosphate pH 7.5, 300 mM NaCl, 1 mM DTT, 1 mM EDTA, 10% glycerol (v/v) as determined by OD_{280}

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 enzyme is not recommended.

REFERENCES: 1) J.J. Wyrick *et al. Biochim. Biophys. Acta* 2012 **1819** 892; 2) B. Zhu *et al. Mol. Cell.* 2005 **20** 601; 3) W.L. Cheung *et al. Cell* 2003 **113** 507; 4) H.C. Beck *et al. Mol. Cell. Proteomics* 2006 **5** 1314; 5) M. Tan *et al. Cell* 2011 **146** 1016; 6) J. Kim *et al. Cell* 2009 **137** 459; 7) L. Wu *et al. Mol. Cell* 2013 doi: 10.1016/j.molcel.2013.01.033. [Epub ahead of print]; 8) L. Wu *et al. Mol. Cell* 2011 **43** 132; 9) S.J. Whitcomb *et al. J. Biol. Chem.* 2012 **287** 23718



Coomassie blue stained SDS-PAGE (16% acrylamide) of 2 µg of purified Histone H2B. MW markers (left) are, from top, 220, 160, 120, 100, 90, 80, 70, 60, **50**, 40, 30, 25, **20**, 15, 10 kDa.



Assay of Histone Acetyltransferases with Histone H2B. The indicated concentrations of either p300 or CBP were assayed as described above in 25 µL reactions, 60 min., 30°C, with 3 µM [^{3}H]-Acetyl-CoA and 5 µM histone H2B as substrates.

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