Histone H3.3

Human, Recombinant

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info@neb.com
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M2507S 00113081508 1

M2507S

100 µg **1.0 mg/ml** **Lot: 0011308**

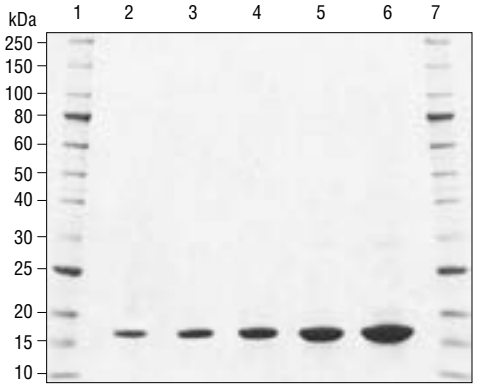
RECOMBINANT **Store at –20°C** **Exp: 8/15**

Description: Histone H3 combines with Histone H4 to form the H3/H4 tetramer. Two H2A/H2B heterodimers interact with an H3/H4 tetramer to form the histone octamer (1,2). It is also modified by various enzymes and can act as a substrate for them. These modifications have been shown to be important in gene regulation.

Histone H3.3, an H3 variant that is found in all eukaryotes from yeast to human, is replication and cell cycle phase independent and is the most common H3 in non-dividing cells (3). It has been shown to be enriched in covalent modifications associated with gene activation (4,5).

Source: An *E. coli* strain that carries a plasmid encoding the cloned human histone H3.3 gene, H3F3A or H3F3B. (Genbank accession number: AK311905)

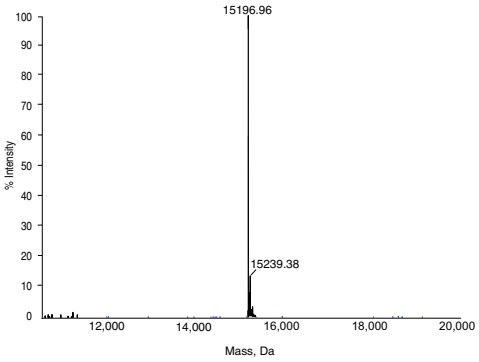
Supplied in: 20 mM Sodium Phosphate (pH 7.0), 300 mM NaCl, 1 mM EDTA and 1 mM DTT.



SDS-PAGE analysis of Histone H3.3 Human, Recombinant. *Lane 1 & 7: NEB Protein Ladder (NEB #P7703), Lane 2 thru 6: 0.5–10.0 µg Histone H3.3 Human, Recombinant (Please see Quality Control section for more information)*

Note: The protein concentration (1 mg/ml, 66 µM) is calculated using the molar extinction coefficient for Histone H3.3 (3960) and its absorbance at 280 nm (6,7). 1.0 A_{280} units = 3.8 mg/ml

Synonym: Histone H3.3A, H3F3, H3.3B.



ESI-TOF Analysis of Histone H3.3 Human, Recombinant.

Quality Control Assays:

SDS-PAGE: 0.5, 1.0, 2.0, 5.0, 10.0 µg of Histone H3.3 Human, Recombinant were loaded on a 10–20% Tris-Glycine SDS-PAGE gel and stained with Coomassie Blue. The calculated molecular weight is 15196.70 Da. Its apparent molecular weight on 10–20% Tris-Glycine SDS-PAGE gel is ~17 kDa.

Mass Spectrometry: The mass of purified Histone H3.3 Human, Recombinant is 15196.96 Da as determined by ESI-TOF MS (Electrospray Ionization-Time of Flight Mass Spectrometry). The average mass calculated from primary sequence is 15196.70 Da. This confirms the protein identity as well as the absence of any modifications of the histone.

N-terminal Protein Sequencing: Protein identity was confirmed using Edman Degradation to sequence the intact protein.

(See other side)

CERTIFICATE OF ANALYSIS

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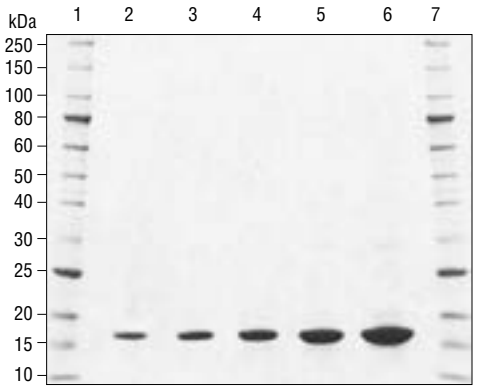

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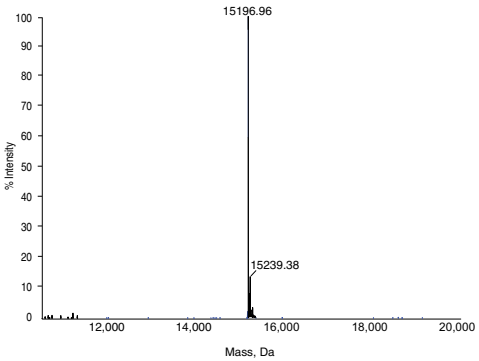

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N-terminal Protein Sequencing: Protein identity was confirmed using Edman Degradation to sequence the intact protein.
(See other side)
CERTIFICATE OF ANALYSIS

Enzyme Modification:

1. G9a Methyltransferase: After incubation of a 25 µl reaction for 10 minutes at 37°C, 1 unit of G9a methyltransferase (NEB #M0235) transfers 0.4 pmols of methyl group to Histone H3.3 Human, Recombinant.
2. SET7 Methyltransferase: After incubation of a 25 µl reaction for 10 minutes at 37°C, 1 unit of SET7 methyltransferase (NEB #M0233) transfers 1 pmols of methyl group to Histone H3.3 Human, Recombinant.

Protease Assay: After incubation of 10 µg of Histone H3.3 Human, Recombinant with a standard mixture of proteins for 2 hours at 37°C, no proteolytic activity could be detected by SDS-PAGE.

Exonuclease Assay: Incubation of a 50 µl reaction containing 10 µg of Histone H3.3 Human, Recombinant with 1 µg of a mixture of single and double-stranded [^{3}H] *E. coli* DNA (200,000 cpm/µg) for 4 hours at 37°C released < 0.1% of the total radioactivity.

Endonuclease Assay: Incubation of a 50 µl reaction containing 10 µg of Histone H3.3 Human, Recombinant with 1 µg of ϕX174 RF I (supercoiled) plasmid DNA for 4 hours at 37°C resulted in < 5.0% conversion to RF II form (nicked circle) as determined by agarose gel electrophoresis.

Protein Sequence: ARTKQTARKSTGGKAPRK QLATKAARKSAPSTGGVKKPHRYRPGTVALREI RRYQKSTELLIRKLPFQRLVREIAQDFKTDLRFQ SAAIGALQEASEAYLVGLFEDTNLCAIHAKRVT IMPKDIQLARRIRGERA (Genbank accession number: P84243)

References:

1. Kornberg, R.D. (1977) *Annu. Rev. Biochem.,* 46, 931–954.
2. van Holde, K.E. (1989) *Chromatin,* 1–497.
3 Gabrielli et al. (1984) *Mol. Cell. Biochem.*, 65, 57-66.
4. Henikoff, S. et al. (2004) *PNAS,* 101, 1525–1530.
5. Hake, S.B. et al (2006) *J.Biol. Chem.,* 281, 559-568.
6. Gill, S.C. and von Hippel, P.H. (1989) *Anal. Biochem.,* 182, 319–326.
7. Pace, C.N. et al. (1995) *Protein Science,* 4, 2411–2423.

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