Histone H3.1 Human, Recombinant

100 µg  1.0 mg/ml  Lot: 0051608
RECOMBINANT Store at –20°C  Exp: 8/18

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 H3.1, an H3 variant that has thus far only been found in mammals, is replication dependent and is associated with gene activation and gene silencing (3).

Source: An E. coli strain that carries a plasmid encoding the cloned human histone H3.1 gene, HIST1H3A or H3FA. (Genbank accession number: AF531274)

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

Note: The protein concentration (1 mg/ml, 65 µM) is calculated using the molar extinction coefficient for Histone H3.1 (4080) and its absorbance at 280 nm (3.4). 1.0 A280 units = 3.8 mg/ml


Quality Control Assays:

SDS-PAGE: 0.5, 1.0, 2.0, 5.0, 10.0 µg of Histone H3.1 Human, Recombinant were loaded on a 10–20% Tris-Glycine SDS-PAGE gel and stained with Coomassie Blue. The calculated molecular weight is 15272.89 Da. Its apparent molecular weight on 10–20% Tris-Glycine SDS-PAGE gel is ~15 kDa.

ESI-TOF Analysis of Histone H3.1 Human, Recombinant.

Deconvoluted Mass (Da)

Intensity Counts (x 10^5)

10,000 15,000 20,000

15,273.35

H3.1 Human, Recombinant is 15273.35 Da

Histone H3.1 (4080) and its absorbance at 280 nm calculated using the molar extinction coefficient for

Mass Spectrometry: The mass of purified Histone H3.1 Human, Recombinant is 15273.35 Da as determined by ESI-TOF MS (Electrospray Ionization-Time of Flight Mass Spectrometry). The average mass calculated from primary sequence is 15272.89 Da. This confirms the protein identity.

Enzyme Modification: SET7 Methyltransferase: After incubation of a 25 µl reaction for 10 minutes at 37°C, 0.2 units of SET7 methyltransferase (NEB #M0233) transfers 50 pmol of methyl group to 2 µM Histone H3.1 Human, Recombinant.

Protease Assay: After incubation of 5 µg of Histone H3.1 Human, Recombinant with a standard mixture of proteins for 4 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 H3.1 Human, Recombinant with 1 µg of a mixture of single and double-stranded [3H] E. coli DNA (200,000 cpm/µg) for 4 hours at 37°C released < 0.1% of the total radioactivity.

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Endonuclease Assay: Incubation of a 50 µl reaction containing 10 µg of Histone H3.1 Human, Recombinant with 1 µg of φX174 RF I (suprecoiled) 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: ARTKOTARKSGKAPRK QLATKAARKSAPATGKVKPPHRYRPGTVALREI RRYQKSTELLIRKLPQRLVREIAQDFKTDLRFO SSAMALQACEAYLGLFEDTNLCAIHAKRTV IMPKDIQLARR/RGERA (Genbank accession number: AAN10051)

Usage Note: When running SDS-PAGE gels, Histone H3.1 can self-oligomerize if freshly prepared DTT (dithiothreitol) is not used when preparing the sample for gel electrophoresis

References: