Histone H3.2 Human, Recombinant

Source: An E. coli strain that carries a plasmid encoding the cloned human histone H3.2 gene, HIST2H3A or HIST2H3C. (Genbank accession number: BC130637)

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, 66 µM) is calculated using the molar extinction coefficient for Histone H3.2 (3960) and its absorbance at 280 nm (4.5). 1.0 A₂₈₀ units = 3.9 mg/ml

Synonym: Histone H3/m, H3/o

Gene Synonym: H3F2, H3FM

Quality Control Assays: SDS-PAGE: 0.5, 1.0, 2.0, 5.0, 10.0 µg of Histone H3 Human, Recombinant were loaded on a 10–20% Tris-Glycine SDS-PAGE gel and stained with Coomassie Blue. The calculated molecular weight is 15256.82 Da. Its apparent molecular weight on 10–20% Tris-Glycine SDS-PAGE gel is ~17 kDa. For a typical example of gel image, please see the product page at www.neb.com.

Mass Spectrometry: The mass of purified Histone H3.2 Human, Recombinant is 15257.09 Da as determined by ESI-TOF MS (Electrospray Ionization-Time of Flight Mass Spectrometry). The average mass calculated from primary sequence is 15256.82 Da. This confirms the protein identity as well as the absence of any modifications of the histone. For a typical example of mass spectrometry data, please see the product page at www.neb.com.

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

Enzyme Modification: SET7 Methyltransferase: After incubation of a 25 µl reaction for 10 minutes at 37°C, 1 unit of SET7 methyltransferase transfers 20 pmols of methyl group to Histone H3.2 Human, Recombinant.

Protease Assay: After incubation of 10 µg of Histone H3.2 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 H3.2 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 radioactive.

Endonuclease Assay: Incubation of a 50 µl reaction containing 10 µg of Histone H3.2 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 Source: ARTKQARKSTGGKAPRKOLA TKARKSAPATGVVKKPHYPRTGVALREIRYYQKS TELLRIKLPQRLVERIAAQDFKTLDFRSSQQMHALGEE SEAYLQLFEDTLNCAHHRVTIPMKDIQDLARRIGE RA (Genbank accession number: Q71D13)

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Histone H3.2, an H3 variant that is found in all eukaryotes except budding yeast, is replication dependent and is associated with gene silencing (3).

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