Histone H3.1/H4 Tetramer Human, Recombinant

1-800-632-7799
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M2509S 002130214021

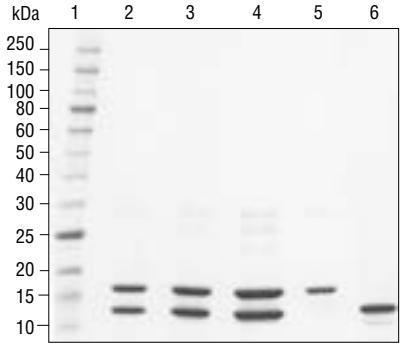
M2509S

1 nmol	**10 µM**	**Lot: 0021302**
RECOMBINANT	**Store at –20°C**	**Exp: 2/14**

Description: Histone H3.1 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). 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). The tetramer 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. Because the histones are folded with their subunit partners, the tetramer may be a better substrate for specific enzymes and modifications (4).

Source: Purified H3.1 and H4 (NEB #M2503 and NEB #M2504) were denatured, refolded and purified by gel filtration.

Supplied in: 20 mM Tris-HCl, pH 8.0 @ 25°C, 2 M NaCl, 1 mM EDTA and 1 mM DTT. Store at –20°C. (Because of high salt, the solution does not always remain frozen.)



SDS-PAGE analysis of Histone H3.1/H4 Tetramer Human, Recombinant.

Lane 1: NEB Protein Ladder (NEB #P7703)
Lane 2–4: 2, 4, 8 µg Histone H3.1/H4 Tetramer
Lane 5: Histone H3.1
Lane 6: Histone H4

Note: To use as a substrate in an enzyme modification assay or other salt sensitive protocol, use 1 to 2 µl of the dimer in a minimum 20 µl reaction so that the salt concentration in the reaction is ≤ 200 mM.

Protein concentration: 10 µM (0.53 mg/ml) calculated using the molar extinction coefficient of Histone H3/H4 Tetremer (21,360) and its absorbance at 280 nm (5,6).

Quality Control Assays:

SDS-PAGE: 2.0, 4.0, 8.0 µg of Histone H3.1/H4 Tetramer Human, Recombinant were loaded on a 10–20% Tris-Glycine SDS-PAGE gel and stained with Coomassie Blue. The calculated molecular weights of the two subunits found in this protein complex are 15,272.89 Da for Histone H3.1 and 11,236.15 Da for Histone H4. Their apparent molecular weight on 10–20% Tris-Glycine SDS-PAGE gel are ~15 kDa and ~11 kDa.

Protease Assay: After incubation of 10 µg (~190 pmol) of Histone H3.1/H4 Tetramer 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 (~190 pmol) of Histone H3.1/H4 Tetramer 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 (~190 pmol) of Histone H3.1/H4 Tetramer 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.

References:

1. Kornberg, R.D. (1977) *Annu. Rev. Biochem.* 46, 931–954.
2. van Holde, K.E. (1989) *Chromatin* 1–497.
3. Hake, S.B. et al. (2006) *J. Biol. Chem.* 281, 559–568.
4. Mersha, F., unpublished observations
5. Gill, S.C. and von Hippel, P.H. (1989) *Anal. Biochem.* 182, 319–326.
6. Pace, C.N. et al. (1995) *Protein Science* 4, 2411–2423.

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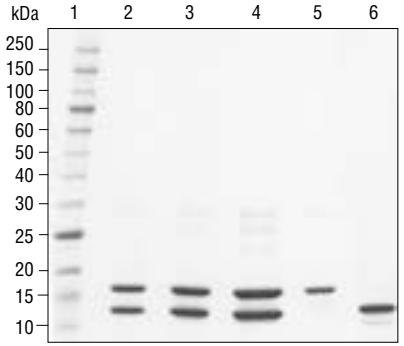

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