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ProductInformation

Anti-Acetyl- & Phospho-Histone H3 [Ac-Lys^{9}, pSer^{10}]
Developed in Rabbit
IgG Fraction of Antiserum

Product Number **H 9161**

Product Description

Anti-Acetyl & Phospho Histone H3 [Ac-Lys^{9}, pSer^{10}] is developed in rabbit using a synthetic, acetylated and phosphorylated [Ac-Lys^{9}, pSer^{10}] histone H3 peptide (amino acids 7-20) corresponding to the N-terminus of human histone H3 conjugated to KLH as immunogen. This histone H3 sequence is identical in many species including mouse, rat, bovine, chicken, frog, drosophila, and *C. elegans*, and is highly conserved (single amino acid substitution) in *Tetrahymena* histone H3. Whole antiserum is fractionated and then further purified by ion-exchange chromatography. The resulting IgG fraction is further purified by absorption on the unmodified histone H3 peptide (human, amino acids 7-20).

Anti-Acetyl & Phospho-Histone H3 [Ac-Lys^{9}, pSer^{10}] recognizes histone H3 acetylated on Lys^{9} and phosphorylated on Ser^{10}. Applications include the detection of [Ac-Lys^{9}, pSer^{10}] histone H3 by immunoblotting (17 kDa) and by indirect immunofluorescence. Staining of [Ac-Lys^{9}, pSer^{10}] histone H3 in immunoblotting is specifically inhibited with the immunizing peptide [Ac-Lys^{9}, pSer^{10}] histone H3 (human, amino acids 7-20). There is no inhibition with non-acetylated and non-phosphorylated human histone H3 peptide.

Histone proteins H3, H4, H2A, and H2B function as building blocks to package eukaryotic DNA into repeating nucleosome units that are folded in higher-order chromatin fibers.[1] The relatively unstructured and highly charged N-terminal tail domains are central to the processes that modulate chromatin structure. A diverse and elaborate array of post-translational modifications including acetylation, phosphorylation, methylation, ubiquitination, and ADP-ribosylation occurs on the N-terminal tail domains of histones.[2,3]

Acetylation of lysine residues within these N-terminal domains by histone acetyl-transferases (HATs), including Gcn5p, P/CAF, p300/CBP and $TAF_{II}250$, is associated with transcriptional activation.[1,4] This modification results in remodeling of the nucleosome structure into an open conformation more accessible to transcription complexes. In most species, histone H3 is primarily acetylated at lysines 9, 14, 18, and 23.[2,5,6] Acetylation at lysine 9 appears to have a dominant role in histone deposition and chromatin assembly in some organisms.[5,7,8]

Phosphorylation of H3, referred to as the nucleosomal response, is localized to a small fraction of highly acetylated H3 and occurs primarily in response to mitogenic and stress stimuli.[2,3,9-12] Phosphorylation of histone H3 on Ser^{10} is tightly correlated with chromosome condensation during both mitosis and meiosis. Phosphorylation at this site is also directly correlated with the induction of immediate-early genes such as *c-jun*, *c-fos*, and *c-myc*.

PKA, Rsk-2, and Msk-1 are necessary for histone H3 phosphorylation.[13-15] Mutations in Rsk-2, associated with Coffin-Lowry syndrome (CLS) in humans and deletion of Rsk-2 in knockout mice, both result in impaired transcriptional activation of *c-fos* and a loss of EGF-induced phosphorylation of H3 *in vivo*.[13,14] The ERK and p38 pathways activate Msk-1 to phosphorylate histone H3.[15]

Reagent

Anti-Acetyl- & Phospho-Histone H3 [Ac-Lys^{9}, pSer^{10}] is supplied as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide.

Precautions and Disclaimer
Due to the sodium azide content, a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazards and safe handling practices.

Storage/Stability
For continuous use, store at 2-8 °C for up to one month. For prolonged storage, freeze in working aliquots at –20 °C. Repeated freezing and thawing is not recommended. Storage in frost-free freezers is also not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilutions should be discarded if not used within 12 hours.

Product Profile
A minimum working dilution of 1:1,000 is determined by immunoblotting using a using a whole cell extract of the mouse fibroblast NIH3T3 cell line treated with sodium butyrate.

A minimum working dilution of 1:1,000 is determined by indirect immunofluorescence using the NIH3T3 mouse fibroblast cell line treated with sodium butyrate.

Note: In order to obtain the best results using different techniques and preparations, we recommend determining the optimal working dilutions by titration.

References

1. Kornberg, R.D., and Lorch, Y., Cell, **98**, 285 (1999).
2. Strahl, B.D., and Allis, C.D., Nature, **403**, 41 (2000).
3. Cheung, P., et al., Cell, **103**, 263 (2000).
4. Struhl, K., Genes Dev., **12**, 599 (1998).
5. Grant, P.A., et al., J. Biol. Chem., **274**, 5895 (1999).
6. Zhang, W., et al., EMBO J., **17**, 3155 (1998).
7. Kuo, M.H., et al., Nature, **383**, 269 (1996).
8. Sobel, R.E., et al., Proc. Natl. Acad. Sci. USA, **92**, 1237 (1995).
9. Mahadevan, L.C., et al., Cell, **65**, 775 (1991).
10. Barratt, M.J., et al., Proc. Natl. Acad. Sci. USA, **91**, 4781 (1994).
11. Thomson, S., et al., Semin. Cell. Biol., **10**, 205 (1999).
12. Chadee, D.N., et al., J. Biol. Chem., **274**, 24914 (1999).
13. Sassone-Corsi, P., et al., Science, **285**, 886 (1999).
14. De Cesare, D., et al., Proc. Natl. Acad. Sci. USA, **95**, 12202 (1998).
15. Thomson, S., et al., EMBO J., **18**, 4779 (1999).

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