3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

Monoclonal Anti-Acetyl & Phospho-Histone H3 (Ac-Lys^{9}, pSer^{10})
Clone APH3-64
Purified Mouse Immunoglobulin

Product Number **H 0788**

Product Description

Monoclonal Anti-Acetyl & Phospho-Histone H3 (Ac-Lys^{9}, pSer^{10}) (mouse IgG2a isotype) is derived from the APH3-64 hybridoma produced by the fusion of mouse myeloma cells (NS1) and splenocytes from BALB/c mice immunized with a synthetic, acetylated and phosphorylated histone H3 peptide (amino acids 7-20, Ac-Lys^{9}, pSer^{1}) corresponding to the N-terminus of human histone H3, conjugated to KLH. 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. The isotype is determined using Sigma ImmunoType™ Kit (Sigma ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Sigma ISO-2).

Monoclonal Anti-Acetyl & Phospho-Histone H3 (Ac-Lys^{9}, pSer^{10}) specifically recognizes human histone H3 only when simultaneously acetylated on Lys^{9} and phosphorylated at Ser^{10}. The antibody may be used in ELISA, immunoblotting (approx. 17 kDa), and immunoprecipitation. Staining of the histone H3 band in immunoblotting is specifically inhibited with the acetylated and phosphorylated human histone H3 immunizing peptide, and is not inhibited with the non-acetylated and non-phosphorylated human histone H3 peptide or by the single phosphorylated or acetylated histone H3 peptide.

Acetylation of histones on lysine residues within the N-terminal domain by histone acetyl-transferase (HATs) including Gcn5p, P/CAF, p300/CBP, and TAFII250 is associated with transcriptional activation.[1, 4] This modification results in remodeling of the nucleosome structure making it 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 histone 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 histone H3 *in vivo*.[13, 14] The ERK and p38 pathways activate Msk-1 to phosphorylate histone H3.[15]

Monoclonal antibodies to acetylated and phosphorylated histone H3 are an important tool for studying chromatin remodeling and gene regulation.

Reagent

Monoclonal 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.

Antibody Concentration: Approx. 2 mg/ml

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 hazardous and safe handling practices.

Storage/Stability

For continuous use, store at 2-8 °C for up to one month. For extended 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 dilution samples should be discarded if not used within 12 hours.

Product Profile
By immunoblotting, a working antibody concentration of 1-2 µg/ml is recommended using a whole cell extract of a Jurkat cell line treated with nocodazole.

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

References

1. Kornberg, R.D., and Lorch, Y., Cell, **98**, 285-294 (1999).
2. Strahl, B.D., and Allis, C.D., Nature, **403**, 41-45 (2000).
3. Cheung, P., et al., Cell, **103**, 263-271 (2000).
4. Struhl, K., Genes Dev., **12**, 599-606 (1998).
5. Grant, P.A., et al., J. Biol. Chem., **274**, 5895-5900 (1999).
6. Zhang, W., et al., EMBO J., **17**, 3155-3167 (1998).
7. Kuo, M.H., et al., Nature, **383**, 269-272 (1996).
8. Sobel, R.E., et al., Proc. Natl. Acad. Sci. USA, **92**, 1237-1241 (1995).
9. Mahadevan, L.C., et al., Cell, **65**, 775-783 (1991).
10. Barratt, M.J., et al., Proc. Natl. Acad. Sci. USA, **91**, 4781-4785 (1994).
11. Thomson, S., et al., Semin. Cell. Biol., **10**, 205-214 (1999).
12. Chadee, D.N., et al., J. Biol. Chem., **274**, 24914-24920 (1999).
13. Sassone-Corsi, P., et al., Science, **285**, 886-891 (1999).
14. De Cesare, D., et al., Proc. Natl. Acad. Sci. USA, **95**, 12202-12207 (1998).
15. Thomson, S., et al., EMBO J., **18**, 4779-4793 (1999).

KAA/EK 11/03