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-PHOSPHO-HISTONE H3 (pSer^{28})
CLONE HTA28
Purified Rat Immunoglobulin

Product Number **H 9908**

Product Description

Monoclonal Anti-Phospho-Histone H3 (pSer^{28}) (rat IgG2a) is derived from the HTA28 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from a WKY/NCrj rat immunized with a synthetic peptide corresponding to amino acids 23-35 (pSer^{28}) of human histone H3, conjugated to KLH.[1] The antibody is purified from culture supernatant of hybridoma cells, grown in a bioreactor.

Monoclonal Anti-Phospho-Histone H3 (pSer^{28}) reacts specifically with histone H3 phosphorylated at Ser28 near its N-terminus, and does not detect the unphosphorylated epitope.[1] It detects the phosphorylated histone molecule at the onset of mitosis (prophase, metaphase and weaker at the beginning of anaphase), but not during late anaphase.[1] The product is reactive in immunoblotting (approx. 15 kDa),[1,2] immunocytochemistry (3.7% formaldehyde-methanol fixation)[1] and flow cytometry. Reactivity has been observed with human,[1] bovine,[1] hamster[1] and mouse.[1,2]

In eukaryotic cells, DNA is associated with histones and other proteins to form chromatin. The cell division cycle constitutes a series of processes that have evolved to create two genetically identical daughter cells from a mother cell. One of these processes is the conversion of relatively amorphous, extended interphase chromatin into condensed, highly ordered mitotic chromosomes. Proper mitotic chromosome condensation is essential for the correct segregation of sister chromatids into two daughter cells.[3] The basic unit of chromatin is the nucleosome core particle, which consists of 140 bp DNA wrapped around an octameric core containing two each of the four conserved core histones: H2A, H2B, H3 and H4. A fifth histone, the linker histone H1, interacts with DNA of variable length, linking adjacent nucleosome cores, and further compacting the chromatin.

Chromatin changes are initiated during G_2 phase of the cell cycle, in preparation for cell division. The most striking morphological change is chromatin condensation, which becomes apparent during prophase and is maximal during the subsequent stages of mitosis. Histone H1 and the N-terminal tail of H3 have key roles in the folding and inter-association of the chromatin fiber.

Two currently known phosphorylation sites are present in the N-terminus of H3; serine-10 and serine-28.[1,2] Mitogenic stimulation, oncogenic transformation, or induction of oncogenic *ras* expression are accompanied with increase in serine-10 phosphorylation of the H3 N-terminal domain.[4] Indeed, it has been shown that phosphorylated H3 is associated with *c-fos* and *c-myc* genes in stimulated cells.[5] Phosphorylation of H3, at both serine-10 and -28, coincides with the induction of mitotic chromosome condensation.[1] H3 phosphorylation may contribute to proto-oncogene induction by modulating chromatin structure and releasing blocks in elongation. In contrast to H1 hyperphosphorylation, site-specific phosphorylation of core histone H3 at serine-10 and -28 appears to occur exclusively during mitosis in mammalian cells. H3 dephosphorylation occurs quite rapidly after mitosis and serine-10/28 remain unphosphorylated throughout the remainder of interphase.[1,5] PP1 has been identified as the H3 phosphatase.[6] Monoclonal antibodies reacting specifically with phosphorylated histone H3, are useful tools to study molecular mechanisms associated with the G_2 to M transition and chromatin condensation, and for the analysis of protein kinase(s) and phosphatase(s) involved in H3 phosphorylation or dephosphorylation. They may also be used in multiparameter analysis to relate H3 phosphorylation in individual cells to the cell's position in the cycle, as well as in relation to expression of other proteins critical for cell cycle.

Reagent
Monoclonal Anti-Phospho-Histone H3 (pSer^{28}) is supplied as a solution in 0.01 M phosphate buffered saline pH 7.4, containing 1% bovine serum albumin and 15 mM sodium azide (see MSDS) as a preservative.

Antibody Concentration: Approx. 0.5 mg/ml.

Precautions and Disclaimer
Due to the sodium azide content a material safety 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 to 8 °C for up to one month. For extended storage, freeze in working aliquots. Repeated freezing and thawing is not recommended. Storage in "frost-free" freezers is 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
A working concentration of 0.5 to 1 µg/ml is determined by immunoblotting using whole extract of cultured human acute T cell leukemia Jurkat cells, treated with Nocodazole.

Note: In order to obtain best results in different techniques and preparations we recommend determining optimal working concentration by titration test.

References

1. Goto, H., et al., J. Biol. Chem., **274**, 25543-25549 (1999).
2. Zhong, S., et al., J. Biol. Chem., **276**, 12932-12937 (2001).
3. Wei, Y., et al., Cell, **97**, 99-109 (1999).
4. Shibata, K., et al., Eur. J. Biochem., **192**, 87-93 (1990).
5. Ajiro, K., et al., J. Biol. Chem., **260**,15379-15381 (1985).
6. Chadee, D.N., et al., J. Biol. Chem., **274**, 24914-24920 (1999).

lpg 7/01