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ProductInformation

Monoclonal Anti-Histone H3, Phosphorylated (Phosphoserine 10)
Clone H3-P
Purified Mouse Immunoglobulin

Product Number **H 6409**

Product Description

Monoclonal Anti-Histone H3, Phosphorylated (pSer10) (mouse IgG2a isotype) is derived from the H3-P hybridoma produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mice immunized with a synthetic peptide corresponding to amino acids 1-18 (pSer10) of human histone H3 conjugated to KLH.[1] The isotype is determined using Sigma ImmunoType™ Kit (Product Code ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Product Code ISO-2).

Monoclonal Anti-Histone H3, Phosphorylated (pSer10), reacts specifically with human[1,2] histone H3 phosphorylated near its N-terminus at serine-10, and does not detect the nonphosphorylated epitope.[1] The antibody may be used for immunoblotting (17 kDa), ELISA,[1] immunocytochemistry[1,2] and flow cytometry.[1,2]

In eukaryotic cells, DNA is associated with histone 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 consisting 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, links adjacent nucleosome cores, and further compacts the chromatin. Chromatin changes are initiated during G2 phase of the cell cycle, in preparation for cell division, and their most striking morphological manifestation 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. Mitogenic stimulation, oncogen transformation, or induction of oncogenic ras expression is accompanied with increases in Ser-10 phosphorylation of the N-terminal domain H3.[4] Indeed, it has been shown that phosphorylated H3 is associated with c-fos and c-myc genes in stimulated cells.[5] H3 phosphorylation may contribute to proto-oncogen induction by modulating chromatin structure and releasing blocks in elongation. PP1 has been identified as the H3 phosphatase.[5] The temporal and spatial patterns of histone H3 phosphorylation implicate a specific role for this modification in mammalian chromosome condensation. In contrast to H1 hyperphosphorylation, site-specific phosphory- lation of core histone H3 at serine 10 (Ser-10) appears to occur exclusively during mitosis in mammalian cells. H3 dephosphorylation occurs quite rapidly after mitosis and Ser-10 remains unphosphorylated throughout the remainder of interphase.[6,7] Phosphorylation of H3 is also seen during premature chromosome condensation (PCC); however, when PCC in interphase nuclei of fused cells is prevented by various metabolic inhibitors, H3 remains dephosphorylated.[1,8] Because the degree of H3 phosphorylation appears to be related to cell differentiation, this feature may have a value as a possible diagnostic and prognostic marker in analyzing the degree of differentiation of acute monocytic leukemias.[2] Monoclonal antibodies reacting specifically with phosphorylated histone H3, are useful tools to study molecular mechanisms associated with the G2 to M transition and chromatin condensation, and for the screening of in vivo inhibitors of kinase(s) or phosphatase(s) involved in H3 phosphorylation or dephosphorylation. They may also be used in multiparameter flow cytometric 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-Histone H3, Phosphorylated (pSer10) is supplied as a solution in 0.01 M phosphate buffered saline pH 7.4, containing 15 mM sodium azide as a preservative.

Antibody Concentration: Approximately 2 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 2-8 °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 100-300 µg/ml is determined by flow cytometry, using cultured human acute T cell leukemia Jurkat cells.

When assayed by flow cytometric analysis (with a FACScan flow cytometer), using 10 µl of antibody at working concentration, to stain $1x10^6$ cells/0.1 ml/test, a fluorescent intensity is observed similar to that obtained with saturating antibody levels. The percent population positive is also at the maximum percent positive using saturating antibody levels.

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

References

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