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

Anti-CENP-A (N-terminal)
produced in rabbit, affinity isolated antibody

Catalog Number **C2493**

Product Description

Anti-CENP-A (N-terminal) is produced in rabbit using as immunogen a synthetic peptide corresponding to amino acids 4-17 located at the N-terminus of human CENP-A (GeneID: 1058), conjugated to KLH. This sequence shows 71% identity in bovine CENP-A. The antibody is affinity-purified using the immunizing peptide immobilized on agarose.

Anti-CENP-A (N-terminal) recognizes human CENP-A (17 kDa) by immunoblotting. Staining of the CENP-A band is specifically inhibited with the immunizing peptide.

The centromere is an essential chromosomal component that is required for proper segregation of chromosomes during mitosis and meiosis. The kinetochore is a large DNA-multiprotein complex that mediates attachment of microtubules to the centromere during mitosis. It comprises both constitutive proteins that are present at the centromere throughout the cell cycle, and transient proteins that are present at various stages. The centromere proteins (CENP) CENP-A, CENP-B and CENP-C are constitutive proteins of the kinetochore. CENP-A (also known as CenH3) is a 17 kDa histone H3-like protein involved in centromeric nucleosome formation.[1, 2] CENP-A is specifically incorporated in direct contact with DNA in the region of the inner kinetochore plate of active centromeres.[3-5] CENP-A has a fold domain similar to histone H3. The N-terminal of CENP-A is 62% identical to histone H3 and is required for targeting to the centromere. In contrast, there is no similarity between the N-terminal (amino acids 1-47) regions of CENP-A and histone H3. CENP-A has been shown to replace histone H3 in nucleosome reconstitution *in vitro*[6] and to be phosphorylated at Ser[7] by both Aurora-A and Aurora-B protein kinases in early mitosis.[7-9] CENP-A phosphorylation is required for proper kinetochore attachment to microtubules and chromosome alignment during mitosis. CENP-A is phosphorylated in early prophase after completion of histone H3 phosphorylation, and is dephosphorylated in early anaphase, before completion of histone H3 dephosphorylation. The temporal differences at G2/M between CENP-A and histone H3 phosphorylation suggest that although they share a similar phosphorylation site, they serve different functions. Knock-out of the CENP-A gene in mouse is lethal.[10] Affected embryos showed severe mitotic problems, including micronuclei and macronuclei formation, nuclear bridging and blebbing, chromatin fragmentation, and hypercondensation. CENP-A depletion results in diffuse CENP-B foci, absence of discrete CENP-C signal on centromeres, and dispersion of CENP-B and CENP-C throughout the nucleus. It has been suggested that CENP-A is essential for kinetochore targeting of CENP-C and plays an early role in organizing centromeric chromatin at interphase.

Reagent

Supplied as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide as a preservative.

Antibody concentration: ~1.5 mg/mL

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet 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, or storage in “frost-free” freezers, is 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

Immunoblotting: a working concentration of 0.5-1 µg/mL is recommended using HEK-293T cells expressing human CENP-A.

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

References

1. Palmer, D.K., et al., *Proc. Natl. Acad. Sci. USA*, **88**, 3734-3738 (1991).
2. Sullivan, K.F., et al., *J. Cell Biol*., **127**, 581-592 (1994).
3. Warburton, P.E., et al., *Curr. Biol*., **7**, 901-904 (1997).
4. Shelby, R.D., et al., *J. Cell Biol*., **136**, 501-513 (1997).
5. Van Hooser, A.A., et al., *J. Cell Sci.*, **114**, 3529-3542 (2001).
6. Yoda, K., et al., *Proc. Natl. Acad. Sci. USA*, **97**, 7266-7271 (2000).
7. Zeitlin, S.G., et al., *J. Cell Sci*., **114**, 653-661 (2000).
8. Zeitlin, S.G., et al., *J. Cell Biol*., **155**, 1147-1157 (2001).
9. Kunitoku, N., et al., *Dev. Cell*, **5**, 853-864 (2003).
10. Howman, E.V., et al., *Proc. Natl. Acad. Sci. USA*, **97**, 1148-1153 (2000).

ER,KAA,PHC 04/07-1