

#2187 Store at -20°C

# Phospho-CENP-A (Ser7) Antibody



- Small 100 µl (10 western blots)
- Petite 40 µl (4 western blots)

**Orders** ■ 877-616-CELL (2355)  
orders@cellsignal.com

**Support** ■ 877-678-TECH (8324)  
info@cellsignal.com

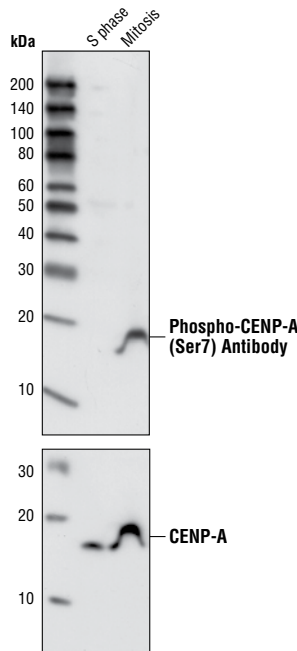
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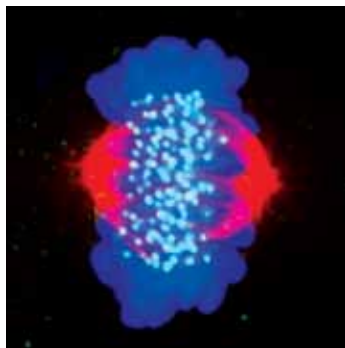
This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W, IP, IF-IC Endogenous	H, (Mk)	17 kDa	Rabbit**

**Background:** Modulation of chromatin structure plays a critical role in the regulation of transcription and replication of the genome in eukaryotes. The nucleosome, made up of four core histone proteins (H2A, H2B, H3, and H4), is the primary building block of chromatin. In addition to the growing number of post-translational histone modifications regulating chromatin structure, cells can also exchange canonical histones with variant histones that can directly or indirectly modulate chromatin structure (1). CENP-A, also known as the chromatin-associated protein CSE4 (capping-enzyme suppressor 4-p), is an essential histone H3 variant that replaces canonical histone H3 in centromeric heterochromatin (2). The greatest divergence between CENP-A and canonical histone H3 occurs in the amino-terminal tail of the protein, which binds linker DNA between nucleosomes and facilitates proper folding of centromeric heterochromatin (3). The amino-terminal tail of CENP-A is also required for recruitment of other centromeric proteins (CENP-C, hSMC1, hZW10), proper kinetochore assembly and chromosome segregation during mitosis (4). Additional sequence divergence in the histone fold domain is responsible for correct targeting of CENP-A to the centromere (5). Many of the functions of CENP-A are regulated by phosphorylation (6,7). Aurora-A-dependent phosphorylation of CENP-A on Ser7 during prophase is required for proper targeting of Aurora-B to the inner centromere in prometaphase, proper kinetochore/microtubule attachment and proper alignment of chromosomes during mitosis (6).



Western blot analysis of extracts from HeLa cells arrested in S phase or mitosis using Phospho-CENP-A (Ser7) Antibody (upper panel) or CENP-A Antibody #2186 (lower panel). S phase cells were treated for 12 hours with thymidine (2 mM final concentration), rinsed three times, released into normal growth medium for 10 hours and then treated an additional 12 hours with thymidine before harvesting. Mitotic cells were treated for 12 hours with thymidine, rinsed three times and then treated for 16 hours with taxol (500 nM final concentration).



Confocal immunofluorescent analysis of a mitotic HeLa cell using Phospho-CENP-A (Ser7) Antibody (green) and  $\beta$ -Tubulin (9F3) Rabbit mAb (Alexa Fluor<sup>®</sup> 555 Conjugate) #2116 (red). Phospho-CENP-A signal is localized to bright spots in the metaphase plate. Blue pseudocolor = DRAQ5<sup>®</sup> #4084 (fluorescent DNA dye).

**Specificity/Sensitivity:** Phospho-CENP-A (Ser7) Antibody detects endogenous levels of human CENP-A protein only when phosphorylated on Ser7. This antibody does not cross-react with other histone proteins, including Histone H3.

**Source/Purification:** Polyclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide (KLH-coupled) corresponding to residues surrounding Ser7 of human CENP-A protein. Antibodies are purified by peptide affinity chromatography.

Entrez-Gene ID #P49450  
Swiss-Prot Acc. #1058

**Storage:** Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at -20°C. Do not aliquot the antibody.

\*Species cross-reactivity is determined by western blot.

\*\*Anti-rabbit secondary antibodies must be used to detect this antibody.

**Recommended Antibody Dilutions:**

Western Blotting	1:1000
Immunoprecipitation	1:25
Immunofluorescence (IF-IC)	1:100
IF Protocol:	Special Protocol Required

For application specific protocols please see the web page for this product at [www.cellsignal.com](http://www.cellsignal.com).

Please visit [www.cellsignal.com](http://www.cellsignal.com) for a complete listing of recommended companion products.

**Background References:**

- Jin, J. et al. (2005) *Trends Biochem Sci* 30, 680–7.
- Ausió, J. (2006) *Brief Funct Genomic Proteomic* 5, 228–43.
- Heit, R. et al. (2006) *Biochem Cell Biol* 84, 605–18.
- Van Hooser, A.A. et al. (2001) *J Cell Sci* 114, 3529–42.
- Black, B.E. et al. (2004) *Nature* 430, 578–82.
- Kunitoku, N. et al. (2003) *Dev Cell* 5, 853–64.
- Zeitlin, S.G. et al. (2001) *J Cell Biol* 155, 1147–57

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**IMPORTANT: For Western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.**

**IMPORTANT: For Immunofluorescence (IF-IC), incubate specimen with diluted antibody in 5% w/v BSA, 1X PBS, 0.3% Triton X-100 at 4°C, overnight.**

**Applications Key:** W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide

**Species Cross-Reactivity Key:** H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish B—bovine

Dg—dog Pg—pig Sc—S. cerevisiae Ce—C. elegans Hr—Horse All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.