#3377 Store at -20°C

Phospho-Histone H3 (Ser10) (D2C8) XP® Rabbit mAb

☑ 100 µl
(10 western blots)

Cell Signaling TECHNOLOGY®

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orders@cellsignal.com
Support ■ 877-678-TECH (8324)
info@cellsignal.com
Web ■ www.cellsignal.com

rev. 07/12/12

For Research Use Only. Not For Use In Diagnostic Procedures.

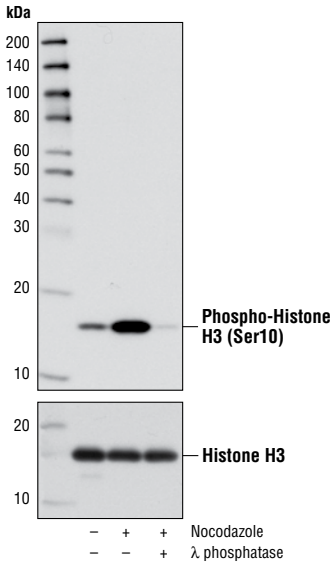
Entrez-Gene ID #8352
Swiss-Prot Acc. #P68431

Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IF-IC, F Endogenous	H, M, R, Mk	17 kDa	Rabbit IgG**

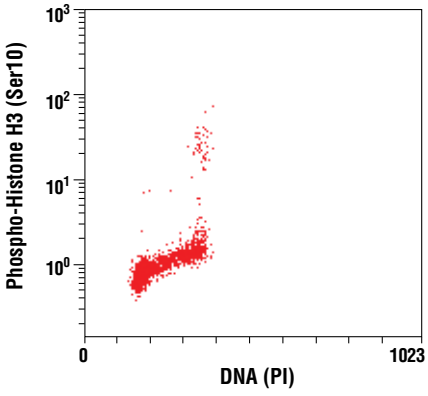
Background: Modulation of chromatin structure plays an important role in the regulation of transcription in eukaryotes. The nucleosome, made up of four core histone proteins (H2A, H2B, H3 and H4), is the primary building block of chromatin (1). The amino-terminal tails of core histones undergo various post-translational modifications, including acetylation, phosphorylation, methylation and ubiquitination (2-5). These modifications occur in response to various stimuli and have a direct effect on the accessibility of chromatin to transcription factors and, therefore, on gene expression (6). In most species, histone H2B is primarily acetylated at Lys5, 12, 15 and 20 (4,7). Histone H3 is primarily acetylated at Lys9, 14, 18 and 23. Acetylation of H3 at Lys9 appears to have a dominant role in histone deposition and chromatin assembly in some organisms (2,3). Phosphorylation at Ser10, Ser28 and Thr11 of histone H3 is tightly correlated with chromosome condensation during both mitosis and meiosis (8-10). Phosphorylation of Thr3 of histone H3 is highly conserved among many species and is catalyzed by the kinase haspin. Immunostaining with phospho-specific antibodies in mammalian cells reveals mitotic phosphorylation of H3 Thr3 in prophase and its dephosphorylation during anaphase (11).

Specificity/Sensitivity: Phospho-Histone H3 (Ser10) (D2C8) XP® Rabbit mAb detects endogenous levels of histone H3 only when phosphorylated at Ser10. The antibody does not cross-react with other phosphorylated histones or with acetylated histones.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic phospho-peptide (KLH-coupled) corresponding to residues surrounding Ser10 of human histone H3.



Western blot analysis of extracts from HeLa cells, either untreated or treated with nocodazole (100 ng/ml for 18 hours), using Phospho-Histone H3 (Ser10) (D2C8) XP® Rabbit mAb #3377 (upper) or Histone H3 Antibody #9715 (lower). Phospho-specificity of the antibody is shown by further treatment of the lysate with λ phosphatase.



Flow cytometric analysis of Jurkat cells using Phospho-Histone H3 (Ser10) (D2C8) XP® Rabbit mAb versus propidium iodide (DNA content). The boxed population indicates Phospho-Histone H3 (Ser10) positive cells.

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.

Storage: Supplied in 10 mM HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. 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
Immunofluorescence (IF-IC)	1:1600
Flow Cytometry	1:1600

For application specific protocols please see the web page for this product at www.cellsignal.com.

Please visit www.cellsignal.com for a complete listing of recommended companion products.

Background References:

(1) Workman, J.L. and Kingston, R.E. (1998) *Annu. Rev. Biochem.* 67, 545-579.
(2) Hansen, J.C. et al. (1998) *Biochemistry* 37, 17637-17641.
(3) Strahl, B.D. and Allis, C.D. (2000) *Nature* 403, 41-45.
(4) Cheung, P. et al. (2000) *Cell* 103, 263-271.
(5) Bernstein, B.E. and Schreiber, S.L. (2002) *Chem. Biol.* 9, 1167-1173.
(6) Jaskelioff, M. and Peterson, C.L. (2003) *Nat. Cell Biol.* 5, 395-399.
(7) Thorne, A.W. et al. (1990) *Eur. J. Biochem.* 193, 701-713.
(8) Hendzel, M.J. et al. (1997) *Chromosoma* 106, 348-360.
(9) Goto, H. et al. (1999) *J. Biol. Chem.* 274, 25543-25549.
(10) Preuss, U. et al. (2003) *Nucleic Acids Res.* 31, 878-885.
(11) Dai, J. et al. (2005) *Genes Dev.* 19, 472-488.

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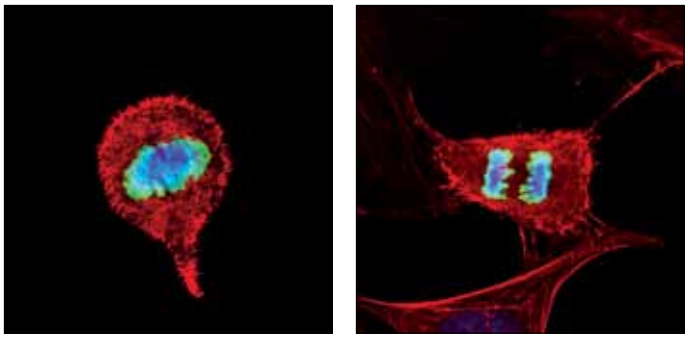
Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide
Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **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.



Confocal immunofluorescent analysis of HeLa cells using Phospho-Histone H3 (Ser10) (D2C8) XP® Rabbit mAb (green). Actin filaments have been labeled with DY-554 phalloidin (red). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).

#4499 Store at -20°C

Histone H3 (D1H2) XP® Rabbit mAb

☐ Small 100 µl
(20 western blots)

☐ Large 300 µl
(60 western blots)

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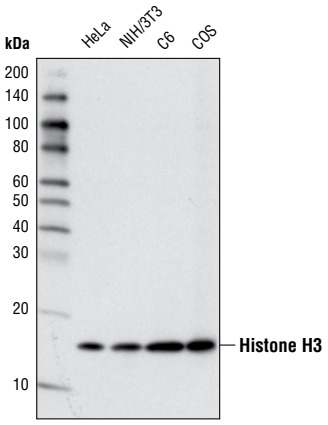
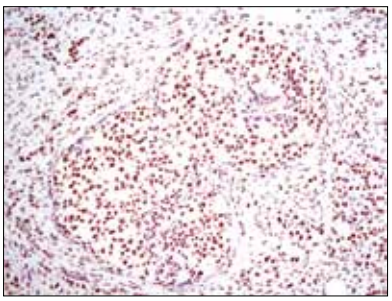
Entrez-Gene ID #8352
Swiss-Prot Acc. #P68431

Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IHC-P, IF-IC Endogenous	H, M, R, Mk, (Hm, C, Dm, X, Z, B)	17 kDa	Rabbit IgG**

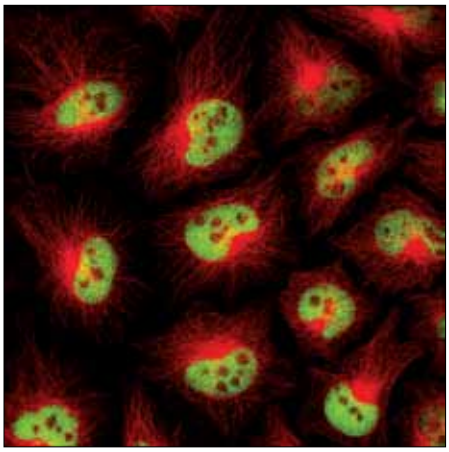
Background: Modulation of chromatin structure plays an important role in the regulation of transcription in eukaryotes. The nucleosome, made up of DNA wound around eight core histone proteins (two each of H2A, H2B, H3 and H4), is the primary building block of chromatin (1). The amino-terminal tails of core histones undergo various post-translational modifications, including acetylation, phosphorylation, methylation and ubiquitination (2-5). These modifications occur in response to various stimuli and have a direct effect on the accessibility of chromatin to transcription factors and, therefore, on gene expression (6). In most species, histone H2B is primarily acetylated at Lys5, 12, 15 and 20 (4,7). Histone H3 is primarily acetylated at Lys9, 14, 18 and 23. Acetylation of H3 at Lys9 appears to have a dominant role in histone deposition and chromatin assembly in some organisms (2,3). Phosphorylation at Ser10, Ser28 and Thr11 of histone H3 is tightly correlated with chromosome condensation during both mitosis and meiosis (8-10). Phosphorylation of Thr3 of histone H3 is highly conserved among many species and is catalyzed by the kinase haspin. Immunostaining with phospho-specific antibodies in mammalian cells reveals mitotic phosphorylation of H3 Thr3 in prophase and its dephosphorylation during anaphase (11).



Western blot analysis of extracts from various cell lines using Histone H3 (D1H2) XP® Rabbit mAb.



Immunohistochemical analysis of paraffin-embedded human breast carcinoma using Histone H3 (D1H2) XP® Rabbit mAb.



Confocal immunofluorescent analysis of HeLa cells using Histone H3 (D1H2) XP®Rabbit mAb (green) and -Tubulin (9F3) Rabbit mAb (Alexa Fluor® 555 Conjugate) #2116 (red).

Specificity/Sensitivity: Histone H3 (D1H2) XP® Rabbit mAb detects endogenous levels of total histone H3 protein. This antibody does not cross-react with other histones.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic peptide (KLH-coupled) derived from the carboxy terminus of the human histone H3 protein.

Storage: Supplied in 10 mM HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. 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	1:2000
Immunohistochemistry (Paraffin)	1:400
Unmasking buffer:	Citrate
Antibody diluent:	SignalStain® Antibody Diluent #8112
Immunofluorescence (IF-IC)	1:200
IF Protocol:	Methanol Permeabilization required

For application specific protocols, please see the web page for this product at www.cellsignal.com

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Background References:

(1) Workman, J.L. and Kingston, R.E. (1998) *Annu Rev Biochem* 67, 545-79.

(2) Hansen, J.C. et al. (1998) *Biochemistry* 37, 17637-41.

(3) Strahl, B.D. and Allis, C.D. (2000) *Nature* 403, 41-5.

(4) Cheung, P. et al. (2000) *Cell* 103, 263-71.

(5) Bernstein, B.E. and Schreiber, S.L. (2002) *Chem Biol* 9, 1167-73.

(6) Jaskelioff, M. and Peterson, C.L. (2003) *Nat Cell Biol* 5, 395-9.

(7) Thorne, A.W. et al. (1990) *Eur J Biochem* 193, 701-13.

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(10) Preuss, U. et al. (2003) *Nucleic Acids Res* 31, 878-85.

(11) Dai, J. et al. (2005) *Genes Dev* 19, 472-88.

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