Store at 4°C

#3458

Phospho-Histone H3 (Ser10) (D2C8) XP® Rabbit mAb (Alexa Fluor® 647 Conjugate)

☑ 100 µl
(50 tests)

New more concentrated formulation

rev. 07/29/11

Cell Signaling TECHNOLOGY®

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Web ■ www.cellsignal.com

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

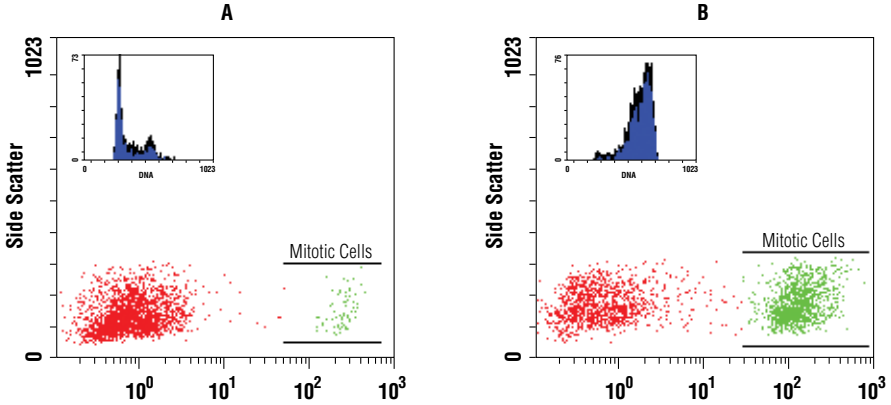
Applications	Species Cross-Reactivity*	Isotype
F, IF-IC	H, M, R, Mk	Rabbit IgG
Endogenous		

Description: This Cell Signaling Technology antibody is conjugated to Alexa Fluor® 647 fluorescent dye and tested in-house for direct flow cytometric analysis in human cells. The unconjugated Phospho-Histone H3 (Ser10) (D2C8) XP® Rabbit mAb #3377 reacts with phospho-histone H3 (Ser10) from human, mouse, rat, and monkey. CST expects that Phospho-Histone H3 (Ser10) (D2C8) XP® Rabbit mAb (Alexa Fluor® 647 Conjugate) will also recognize phospho-histone H3 (Ser10) in these species.

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 (Alexa Fluor® 647 Conjugate) 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 phosphopeptide corresponding to residues surrounding Ser10 of human histone H3. This antibody was conjugated to Alexa Fluor® 647 under optimal conditions with an F/P ratio of 2-6. The Alexa Fluor® 647 dye is maximally excited by red light (e.g. 633 nm He-Ne laser). Antibody conjugates of the Alexa Fluor® 647 dye produce bright far-red-fluorescence emission, with a peak at 665 nm.



Phospho-Histone H3 (Ser10) (Alexa Fluor® 647 Conjugate)

Flow cytometric analysis of Jurkat cells, untreated (A) or taxol-treated (B), stained with Phospho-Histone H3 (Ser10) (D2C8) XP® Rabbit mAb (Alexa Fluor® 647 Conjugate). The blue inserts represent PI (DNA) staining alone, showing an increase in the number of mitotic cells in the taxol-treated sample.

Storage: Supplied in PBS (pH 7.2), less than 0.1% sodium azide, 2 mg/ml BSA. Store at 4°C. *Protect from light. Do not freeze.*

***Species cross-reactivity other than human is determined by immunofluorescence using the unconjugated antibody.**

Recommended Antibody Dilutions:

Flow Cytometry	1:50
Immunofluorescence (IF-IC)	1:400

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.

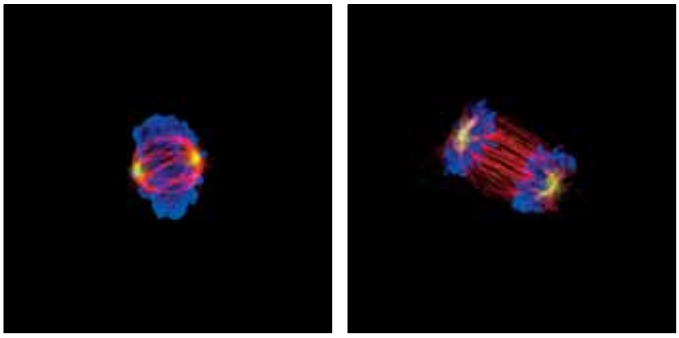
(9) Goto, H. et al. (1999) *J. Biol. Chem.* 274, 25543–25549.

(10) Preuss, U. et al. (2003) *Nucleic Acids Res.* 31, 878–885.

(11) Dai, J. et al. (2005) *Genes Dev.* 19, 472–488.

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



Confocal immunofluorescent analysis of HeLa cells using Phospho-Histone H3 (Ser10) (D2C8) XP® Rabbit mAb (Alexa Fluor® 647 Conjugate) (blue pseudocolor), β-Tubulin (9F3) Rabbit mAb (Alexa Fluor® 555 Conjugate) #2116 (red) and Phospho-Aurora A (Thr288)/Aurora B (Thr232)/Aurora C (Thr198) (D13A11) XP® Rabbit mAb #2914 (green).