

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

✓ 500 µl  
(50 tests)

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This product is for *in vitro* research use only and is not intended for use in humans or animals.  
This product is not intended for use as a therapeutic or in diagnostic procedures.

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

Applications	Species Cross-Reactivity*	Molecular Wt.	Source
F Endogenous	H, M	17 kDa	Rabbit

**Description:** This Cell Signaling Technology Antibody was conjugated to Alexa Fluor® 647 fluorescent dye and tested in-house for direct Flow Cytometry in human and mouse cells. The unconjugated antibody, #9701 reacts with phospho-histone H3 (Ser10) from human, mouse, rat, and monkey. CST expects that Phospho-Histone H3 (Ser10) Antibody (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 (2,3). 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) Antibody (Alexa Fluor® 647 Conjugate) detects endogenous levels of histone H3 only when phosphorylated at serine 10. The antibody does not cross-react with other phosphorylated histones or with acetylated histones.

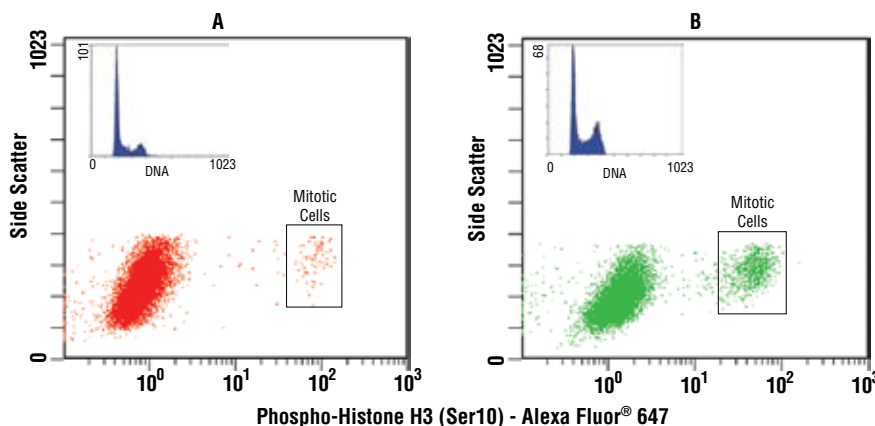
**Source/Purification:** Polyclonal antibodies are produced by immunizing rabbits with a synthetic phospho-peptide corresponding to residues surrounding Ser10 of human histone H3. Antibodies are purified by protein A and peptide affinity chromatography. The antibody was conjugated to Alexa Fluor® 647 under optimal conditions with an F/P ratio of 2-6.

**Storage:** Supplied in PBS (pH 7.2), 0.1% Sodium azide, 2.0 mg/ml BSA. Store at 4°C. Protect from light. Do not freeze.

**Recommended Antibody Dilutions:ns:**  
Flow Cytometry 1:10

## 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.



Flow cytometric analysis of THP-1 cells, untreated (A) or paclitaxel-treated (B), stained with Phospho-Histone H3 (Ser10) Antibody (Alexa Fluor® 647 Conjugate). The blue inserts represent PI (DNA) staining alone, showing an increase in the number of mitotic cells in the paclitaxel-treated sample.

The Alexa Fluor® dye antibody conjugates in this product are sold under license from Molecular Probes, Inc., for research use only, except for use in combination with DNA microarrays. The Alexa Fluor® dyes (except for Alexa Fluor® 430 dye) are covered by pending and issued patents.

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**Applications Key:** W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E—ELISA 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—zebra fish B—bovine  
Dg—Dog Pg—Pig Sc—S. cerevisiae All—all species expected Species enclosed in parentheses are predicted to react based on 100% sequence homology.

## Flow Cytometry Protocol for Intracellular Staining Using Conjugated Primary Antibodies

### A Solutions and Reagents

1. **1X Phosphate Buffered Saline (PBS):** Dissolve 8 g NaCl, 0.2 g KCl, 1.44 g  $\text{Na}_2\text{HPO}_4$  and 0.24 g  $\text{KH}_2\text{PO}_4$  in 800 ml distilled water ( $\text{dH}_2\text{O}$ ). Adjust the pH to 7.4 with HCl and the volume to 1 liter. Store at room temperature.
2. Formaldehyde (methanol free)
3. **Incubation Buffer:** Dissolve 0.5 g bovine serum albumin (BSA) in 100ml 1X PBS. Store at 4°C

### B Fixation

1. Collect cells by centrifugation and aspirate supernatant.
2. Resuspend cells briefly in 0.5-1 ml PBS. Add formaldehyde to a final concentration of 2-4% formaldehyde.
3. Fix for 10 minutes at 37°C.
4. Chill tubes on ice for 1 minute.

### C Permeabilization

1. Permeabilize cells by adding ice-cold 100% methanol slowly to pre-chilled cells, while gently vortexing, to a final concentration of 90% methanol. Alternatively, to remove fix prior to permeabilization, pellet cells by centrifugation and resuspend in 90% methanol.
2. Incubate 30 minutes on ice.
3. Proceed with staining or store cells at -20°C in 90% methanol.

### D Staining Using Conjugated Primary Antibodies

**NOTE:** Allow for isotype matched controls for monoclonal antibodies or species matched IgG for polyclonal antibodies. Count cells using a hemacytometer or alternative method.

1. Aliquot  $5 \times 10^5$  cells into each assay tube (by volume).
2. Add 2-3 ml Incubation Buffer to each tube and rinse by centrifugation.
3. Resuspend cells in 90  $\mu\text{l}$  Incubation Buffer per assay tube.
4. Block in Incubation Buffer for 10 minutes at room temperature.
5. Add 10  $\mu\text{l}$  of conjugated antibody to the assay tubes.
6. Incubate for 30-60 minutes, in the dark at room temperature.
7. Rinse as before in Incubation Buffer by centrifugation.
8. Resuspend cells in 0.5 ml PBS and analyze on flow cytometer.