

Phospho-Histone H3 (Ser10) Antibody (Alexa Fluor® 488 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.

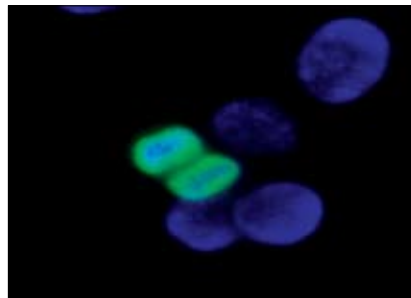
Applications	Species Cross-Reactivity	Molecular Wt.	Source
IF-IC, F	H, M	17 kDa	Rabbit

Description: This Cell Signaling Technology Antibody was conjugated to Alexa Fluor® 488 fluorescent dye and tested in-house for direct Flow Cytometry and Immunofluorescence 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® 488 Conjugate) will also recognize phospho-histone H3 (Ser10) in these species.

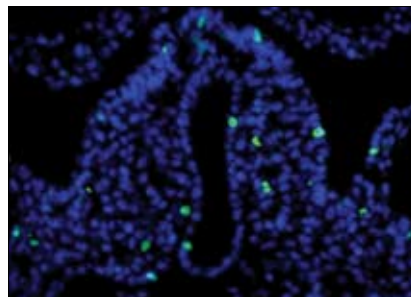
Background: Modulation of chromatin structure plays an important role in the regulation of transcription in eukaryotes. The nucleosome, made up of four core histone proteins (H2A, H2B, H3 and H4), is the primary building block of chromatin (1). The amino-terminal tails of core histones undergo various 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® 488 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.

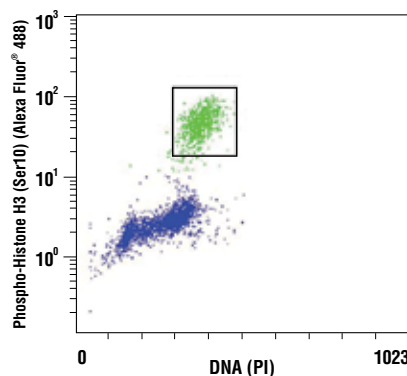
Source/Purification: Polyclonal antibodies are produced by immunizing rabbits with a synthetic phospho-peptide (KLH-coupled) corresponding to residues surrounding Ser10 of human histone H3. Antibodies are purified by protein A and peptide affinity chromatography. The antibody was conjugated to Alexa Fluor®488 under optimal conditions with an F/P ratio of 2-6.



Immunocytochemical staining of untreated NIH/3T3 cells, using Phospho-Histone H3 (Ser10) Antibody (Alexa Fluor®488 conjugate) (green) and DAPI (blue).



Staining of a transverse section of an E9.5 mouse embryo showing the primitive gut endoderm using Phospho-Histone H3 (Ser10) Antibody (Alexa Fluor® 488 conjugate)(green) and DAPI (blue). (Image provided by Dr. Roque Bort)



◀ Flow cytometric analysis of THP1 cells treated with paclitaxel, using Phospho-Histone H3 (Ser10) Antibody (Alexa Fluor®488 conjugate) versus propidium iodide (DNA content). The box indicates phospho-histone H3 positive cells.

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

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.*

Recommended Antibody Dilutions:

Flow Cytometry 1:10
Immunofluorescence (IF-IC) 1:40

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.

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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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 Na_2HPO_4 and 0.24 g KH_2PO_4 in 800 ml distilled water (dH_2O). 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 5×10^5 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 μl Incubation Buffer per assay tube.
4. Block in Incubation Buffer for 10 minutes at room temperature.
5. Add 10 μl 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.

Immunofluorescence Protocol

***IMPORTANT:** Please refer to the **APPLICATIONS** section on the front page of the data sheet to determine **IF THIS PRODUCT** is validated and approved for the specific protocol you will be using.

A Solutions and Reagents

NOTE: Prepare solutions with Milli-Q or equivalently purified water.

- 1. 10X Phosphate Buffered Saline (PBS):** To prepare 1 L add 80 g sodium chloride (NaCl), 2 g potassium chloride (KCl), 14.4 g sodium phosphate, dibasic (Na₂HPO₄) and 2.4 g potassium phosphate, monobasic (KH₂PO₄) to 1 L dH₂O. Adjust pH to 7.4.
- 2. Formaldehyde, 16%, methanol free,** Polysciences, Inc. (cat# 18814), use fresh, store opened vials at 4°C in dark, dilute in PBS for use.
- 3. Xylene**
- 4. Ethanol, anhydrous denatured, histological grade, 100% and 95%**
- 5. Distilled water (dH₂O)**
- 6. Blocking Buffer:** To prepare 25 mL, add 2.5 mL 10X PBS, 1.25 mL normal serum from the same species as the secondary antibody (eg. normal goat serum, normal donkey serum) and 21.25 mL dH₂O and mix well. While stirring, add 75 µL Triton X-100 (100%).
- 7. Antibody Dilution Buffer:** To prepare 40 mL, add 4 mL 10X PBS to 36 mL dH₂O, mix. Add 0.4 g BSA and mix well. While stirring, add 120 µL Triton X-100 (100%).
- 8. 10 mM Sodium Citrate Buffer:** To prepare 1 L, add 2.94 g sodium citrate trisodium salt dihydrate (C₆H₅Na₃O₇•2H₂O) to 1 L dH₂O. Adjust pH to 6.0.
- 9. 1X PBS, high salt (0.4M) (high salt PBS):** To prepare 1L, add 100 ml 10X PBS to 900 ml dH₂O. Add 23.38 g NaCl and mix.
- 10. Fluorochrome-conjugated secondary antibody**

NOTE: When using any primary or fluorochrome-conjugated secondary antibody for the first time, titrate the antibody to determine which dilution allows for the strongest specific signal with the least background for your sample.

- 11. Prolong® Gold Antifade Reagent** (Invitrogen, Eugene, OR, Cat# P36930)

B Specimen Preparation

I. Cultured Cell Lines (IF-IC)

IMPORTANT: Please check the **APPLICATIONS** section of the data sheet to verify that this product is validated and approved for **(IF-IC)**.

NOTE: Cells should be grown, treated, fixed, and stained directly in multiwell plates, chamber slides, or on coverslips.

- 1. Rinse cells briefly in PBS.**
- 2. Aspirate PBS, cover cells to a depth of 2-3 mm with 2-4% formaldehyde in PBS.**

NOTE: Formaldehyde is toxic, use only in fume hood.

- 3. Allow cells to fix for 15 minutes at room temperature.**
- 4. Aspirate fixative, rinse three times in PBS for 5 minutes each.**
- 5. Methanol Permeabilization Step (if required, please refer to front page):** After formaldehyde fixation, cover cells with ice-cold 100% methanol (use enough to cover cells completely to a depth of 3-5 mm, DO NOT LET CELLS DRY), incubate cells in methanol for 10 minutes at -20°C, rinse in PBS for 5 minutes.
- 6. Proceed with Immunostaining section C.**

II. Paraffin Sections (IF-P)

IMPORTANT: Please check the **APPLICATIONS** section of the data sheet to verify that this product is validated and approved for **(IF-P)**.

Deparaffinization/Rehydration:

- 1. Incubate sections in three washes of xylene for 5 minutes each.**
- 2. Incubate sections in two washes of 100% ethanol for 10 minutes each.**
- 3. Incubate sections in two washes of 95% ethanol for 10 minutes each.**
- 4. Rinse sections twice in dH₂O for 5 minutes each.**

Antigen Unmasking:

- 1. Place slides in room temperature 10 mM sodium citrate buffer pH 6.0.**
- 2. Bring slides to boiling in sodium citrate buffer using water bath or microwave, then maintain at 95-99°C for 10 minutes.**
- 3. Cool slides for 30 minutes on bench top.**
- 4. Rinse sections in dH₂O three times for 5 minutes each.**
- 5. Rinse sections in PBS for 5 minutes.**
- 6. Proceed with Immunostaining section C.**

III. Frozen/Cryostat Sections (IF-F)

IMPORTANT: Please check the **APPLICATIONS** section of the data sheet to verify that this product is validated and approved for **(IF-F)**.

NOTE: Fresh frozen/unfixed sections should be fixed immediately in 2-4% formaldehyde as follows to preserve signaling epitopes.

- 1. Cover sections with 2-4% formaldehyde in PBS**

NOTE: Formaldehyde is toxic, use only in fume hood.

- 2. Allow sections to fix for 15 minutes at room temperature.**
- 3. Rinse slides three times in PBS for 5 minutes each.**

C Immunostaining

NOTE: All subsequent incubations should be carried out at room temperature unless otherwise noted in a humid light-tight box or covered dish/plate to prevent drying and fluorochrome fading.

- 1. Block specimen in Blocking Buffer for 60 minutes.**
- 2. While blocking, prepare primary antibody by diluting as indicated on datasheet in Antibody Dilution Buffer.**
- 3. Aspirate blocking solution, apply diluted primary antibody.**

NOTE: For double-labeling, prepare a cocktail of the primary antibodies at their appropriate dilution in Antibody Dilution Buffer.

- 4. Incubate overnight at 4°C.**
- 5. Rinse three times in PBS for 5 minutes each.**

OPTION: To decrease background stain, rinse in high salt PBS for two minutes between second and third PBS rinses. Be aware, this may reduce specific staining of some antibodies.

NOTE: If using primary antibodies directly conjugated with Alexa Fluor® fluorochromes, then skip to step C8.

- 6. Incubate specimen in fluorochrome-conjugated secondary antibody diluted in Antibody Dilution Buffer for 1-2 hours at room temperature in dark.**

NOTE: For double-labeling, prepare a cocktail of fluorochrome-conjugated secondary antibodies at their appropriate dilutions in Antibody Dilution Buffer.

- 7. Rinse in PBS/high salt PBS as in step 5.**
- 8. Coverslip slides with Prolong® Gold Antifade Reagent or apply just enough to cover cells in multiwell plate.**
- 9. Seal slides by painting around edges of coverslips with nail polish.**
- 10. For best results examine specimens immediately using appropriate excitation wavelength. For long term storage, store slides flat at 4°C protected from light.**