

#9719 Store at 4°C

Phospho-Histone H2A.X (Ser139) (20E3) Rabbit mAb (Alexa Fluor® 488 Conjugate)

✓ 500 µl (50 tests)



Orders ■ 877-616-CELL (2355) orders@cellsignal.com
Support ■ 877-678-TECH (8324) info@cellsignal.com
Web ■ www.cellsignal.com

rev. 06/02/08

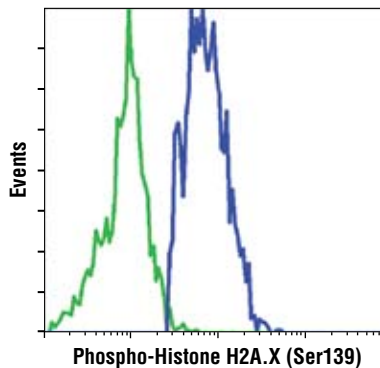
This product is for *in vitro* research use only and is not intended for use in humans or animals.

Applications	Species Cross-Reactivity	Source	Isotype
IF-IC, F Endogenous	H, M*	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 and methylation (2-4). These modifications occur in response to cell signaling stimuli and have a direct effect on gene expression. DNA damage caused by ionizing radiation, UV-light, or radiomimetic agents results in rapid phosphorylation of the histone H2A family member H2A.X at Ser139 by ATM (5,6). Within minutes following DNA damage, Ser139-phosphorylated H2A.X localizes to sites of DNA damage at subnuclear foci (7).

Description: This Cell Signaling Technology Antibody was conjugated to Alexa Fluor® 488 fluorescent dye and tested in-house for direct flow cytometric analysis of human cells. The unconjugated antibody #9718 reacts with human and mouse Phospho-Histone H2A.X. CST expects that Phospho-Histone H2A.X (Ser139) (20E3) Rabbit mAb (Alexa Fluor® 488 conjugate) will also recognize Phospho-Histone H2A.X in these species*.

Directions for Use: Add 10 µl of the conjugated antibody to 500,000 cells in 90 µl PBS/0.5% BSA. See protocol for more details.



Flow cytometric analysis of Jurkat cells, untreated (green) or etoposide-treated (blue), using Phospho-Histone H2A.X (Ser139) (20E3) Rabbit mAb (Alexa Fluor® 488 Conjugate).

Specificity/Sensitivity: Phospho-Histone H2A.X (Ser139) (20E3) Rabbit mAb detects endogenous levels of H2A.X only when phosphorylated at serine 139.

Source/Purification: Monoclonal antibodies are produced by immunizing rabbits with a synthetic phospho-peptide (KLH-coupled) corresponding to residues surrounding Ser139 of human H2A.X. The antibody was conjugated to Alexa Fluor® 488 under optimal conditions with an F/P ratio of 2-5.

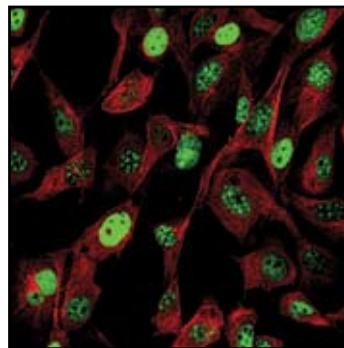
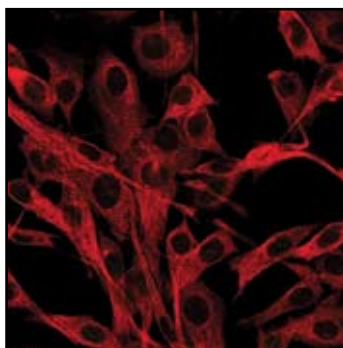
Entrez-Gene ID #3014
Swiss-Prot Acc. #P16104

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

Recommended Antibody Dilutions:
Immunofluorescence (IF-IC) 1:10
Flow Cytometry 1:10

Companion Products:
Phospho-Histone H2A.X (Ser139) Antibody #2577
H2A.X Antibody #2595
Phospho-Histone H3 (Ser10) Antibody (Alexa Fluor® 647 Conjugate) #9716

- 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) Cheung, P. et al. (2000) *Cell* 103, 263-271.
 - (4) Thorne, A.W. et al. (1990) *Eur. J. Biochem.* 193, 701-713.
 - (5) Rogakou, E.P. et al. (1998) *J. Biol. Chem.* 273, 5858-5868.
 - (6) Burma, S. et al. (2001) *J. Biol. Chem.* 276, 42462-42467.
 - (7) Rogakou, E.P. et al. (1999) *J. Cell Biol.* 146, 905-916.



Confocal immunofluorescent images of NIH/3T3 cells, untreated (left) or etoposide-treated (right), double-labeled with Phospho-Histone H2A.X (Ser139) (20E3) Rabbit mAb (Alexa Fluor® 488 Conjugate) (green) and β-Tubulin Antibody #2146 (red).

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. Alexa Fluor® is a registered trademark of Molecular Probes, Inc.

© 2008 Cell Signaling Technology, Inc. Selected Rabbit Monoclonals are produced under license (granting certain rights, including those under U.S. Patent No. 5,675,063) from Epitomics, Inc.

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.

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.

- 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 (Na_2HPO_4) and 2.4 g potassium phosphate, monobasic (KH_2PO_4) to 1 L dH_2O . Adjust pH to 7.4.
- Formaldehyde, 16%, methanol free, Polysciences, Inc. (cat# 18814), use fresh, store opened vials at 4°C in dark, dilute in PBS for use.
- Xylene
- Ethanol, anhydrous denatured, histological grade, 100% and 95%
- Distilled water (dH_2O)
- 1X PBS/0.3% Triton X-100 (PBS/Triton):** To prepare 1 L, add 100 ml 10X PBS to 900 ml dH_2O . Add 3 ml Triton X-100 and mix.
- 10 mM Sodium Citrate Buffer:** To prepare 1 L, add 2.94 g sodium citrate trisodium salt dihydrate ($\text{C}_6\text{H}_5\text{Na}_3\text{O}_7 \cdot 2\text{H}_2\text{O}$) to 1 L dH_2O . Adjust pH to 6.0.
- 1X PBS, high salt (0.4M) (high salt PBS):** To prepare 1L, add 100 ml 10X PBS to 900 ml dH_2O . Add 23.38 g NaCl and mix.
- 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.

- Vectashield Mounting Medium (Vector Labs, Burlingame, CA, cat# H-1000) or Vectashield Mounting Medium with DAPI (cat# H-1200)

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: This general fixation protocol will work with most antibodies and cell lines. However, we recommend you try different IF/IC fixation methods (methanol or acetone alone, aldehyde alone, or combinations of these) to identify the optimal fixation protocol for each antibody and/or cell line.

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

- Rinse cells briefly in PBS.
- 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.

- Allow cells to fix for 15 minutes at room temperature.
- Aspirate fixative, rinse three times in PBS for 5 minutes each.

OPTION: 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 in freezer, rinse in PBS for 5 minutes.

- 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:

- Incubate sections in three washes of xylene for 5 minutes each.

- Incubate sections in two washes of 100% ethanol for 10 minutes each.
- Incubate sections in two washes of 95% ethanol for 10 minutes each.
- Rinse sections twice in dH_2O for 5 minutes each.

Antigen Unmasking:

- Place slides in room temperature 10 mM sodium citrate buffer pH 6.0.
- Bring slides to boiling in sodium citrate buffer using water bath or microwave, then maintain at 95-99°C for 10 minutes.
- Cool slides for 30 minutes on bench top.
- Rinse sections in dH_2O three times for 5 minutes each.
- Rinse sections in PBS for 5 minutes.
- 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.

- Cover sections with 2-4% formaldehyde in PBS

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

- Allow cells to fix for 15 minutes at room temperature.
- 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.

- Block specimen in 5% normal serum from same species as secondary antibody (eg. normal goat serum, normal donkey serum) in PBS/Triton for 60 minutes.
- While blocking, prepare primary antibody by diluting as indicated on datasheet in PBS/Triton. You will need 50-100 μl per section, 25-50 μl per coverslip, chamber, or well (48 or 96 well plate).
- Aspirate blocking solution, apply diluted primary antibody.

NOTE: For double-labeling, prepare a cocktail of mouse and rabbit primary antibodies at their appropriate dilutions in PBS/Triton.

- Incubate overnight at 4°C.
- 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 AlexaFluor® fluorochromes, then skip to step C8.

- Incubate in fluorochrome-conjugated secondary antibody diluted in PBS/Triton for 1-2 hours at room temperature in dark.

NOTE: For double-labeling, prepare a cocktail of fluorochrome-conjugated anti-mouse and anti-rabbit primary antibodies at their appropriate dilutions in PBS/Triton.

- Rinse in PBS/high salt PBS as in step 5.
- Coverslip slides with Vectashield Mounting Medium or apply just enough to cover cells in multiwell plate.
- Seal slides by painting around edges of coverslips with nail polish.
- Examine specimens immediately using appropriate excitation wavelength, depending on fluorochrome for best results or store flat at 4°C in dark.

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.