Purified Mouse anti-H2AX (pS139)

**Product Information**

**Material Number:** 560443  
**Alternate Name:** H2A.X; H2A/X; H2AFX; HIST5-2AX; gamma-H2AX; γ-H2AX; H2AX (pS140)  
**Size:** 0.1 mg  
**Concentration:** 0.5 mg/ml  
**Clone:** N1-431  
**Immunogen:** Phosphorylated Human H2AX Peptide  
**Isotype:** Mouse (BALB/c) IgG1, κ  
**Reactivity:**  
- **QC Testing:** Human  
- **Tested in Development:** Mouse  
**Storage Buffer:** Aqueous buffered solution containing ≤0.09% sodium azide.

**Description**

Histones are highly basic proteins that complex with DNA to form chromatin. The H2AX histone (~15 kDa calculated molecular weight) is a member of the H2A histone family whose members are components of nucleosomal histone octamers. Double-stranded breaks in DNA caused by replication errors, apoptosis, or other physiological processes (including, immunoglobulin and TCR gene recombinations) and DNA damage caused by ionizing radiation, UV light, or cytotoxic agents lead to phosphorylation of H2AX on serine 139. H2AX (pS139) is also referred to as H2AX (pS140) when the N-terminal methionine that is normally excised during posttranslational processing is included in amino acid sequence numbering. Kinases such as ataxia telangiectasia mutated (ATM) or ATM-Rad3-related (ATR) phosphorylate H2AX to induce its function. Phosphorylated H2AX (also termed, gamma-H2AX) functions to recruit and localize DNA repair proteins or cell cycle checkpoint factors to the DNA-damaged sites. In this way, phosphorylated H2AX promotes DNA repair and maintains genomic stability and thus helps prevent oncogenic transformations. Immunofluorescent staining and bioimaging analysis of cultured cells can be used to readily identify H2AX (pS139)-containing foci. As such, H2AX (pS139) immunofluorescence localization serves as a biomarker for nuclear sites of DNA damage (e.g., double-stranded DNA breaks) in affected cells.

**Preparation and Storage**

Store undiluted at 4°C.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

**Application Notes**

**Application**

- Western blot Routinely Tested  
- Bioimaging Tested During Development

**BD Biosciences**

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Recommended Assay Procedure:

Recommended Assay Procedure for Bioimaging:
http://www.bdbiosciences.com/support/resources/protocols/certified_reagents.jsp or http://www.bdbiosciences.com/bioimaging/reagents

1. Seed the cells in appropriate culture medium at an appropriate cell density in a BD Falcon™ 96-well Imaging Plate (Cat. No. 353219), and culture overnight to 48 hours.

2. Remove the culture medium from the wells, and wash (one to two times) with 100 µl of 1× PBS.

3. Fix the cells by adding 100 µl of fresh 3.7% Formaldehyde in PBS or BD Cytofix™ fixation buffer (Cat. No. 554655) to each well and incubating for 10 minutes at room temperature (RT).

4. Remove the fixative from the wells, and wash the wells (one to two times) with 100 µl of 1× PBS.

5. Permeabilize the cells using either cold methanol (a), Triton™ X-100 (b), or Saponin (c):
   a. Add 100 µl of -20°C 90% methanol or -20°C BD™ Phosflow Perm Buffer III (Cat. No. 558050) to each well and incubate for 5 minutes at RT.
   b. Add 100 µl of 0.1% Triton™ X-100 to each well and incubate for 5 minutes at RT.
   c. Add 100 µl of 1× Perm/Wash buffer (Cat. No. 554723) to each well and incubate for 15 to 30 minutes at RT. Continue to use 1× Perm/Wash buffer for all subsequent wash and dilutions steps.

6. Remove the permeabilization buffer from the wells, and wash one to two times with 100 µl of appropriate buffer (either 1× PBS or 1× Perm/Wash buffer, see step 5.c.).

7. Optional blocking step: Remove the wash buffers, and block the cells by adding 100 µl of blocking buffer BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656) or 3% FBS in appropriate dilution buffer to each well and incubating for 15 to 30 minutes at RT.

8. Dilute the antibody to its optimal working concentration in appropriate dilution buffer. Titrate purified (unconjugated) antibodies and second-step reagents to determine the optimal concentration. If using a Bioimaging Certified antibody conjugate, dilute it 1:10.

9. Add 50 µl of diluted antibody per well and incubate for 60 minutes at RT. Incubate in the dark if using fluorescently labeled antibodies.

10. Remove the antibody, and wash the wells three times with 100 µl of wash buffer. An optional detergent wash (100 µl of 0.05% Tween in 1× PBS) can be included prior to the regular wash steps.

11. If the antibody being used is fluorescently labeled, then move to step 12. Otherwise, if using a purified unlabeled antibody, repeat steps 8 to 10 with a fluorescently labeled second-step reagent to detect the purified antibody.

12. After the final wash, counter-stain the nuclei by adding 100 µl of a 2 µg/ml solution of Hoechst 33342 (eg, Sigma-Aldrich Cat. No. B2261) in 1× PBS to each well at least 15 minutes before imaging.

13. View and analyze the cells on an appropriate imaging instrument.

Suggested Companion Products

<table>
<thead>
<tr>
<th>Catalog Number</th>
<th>Name</th>
<th>Size</th>
<th>Clone</th>
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<tbody>
<tr>
<td>554002</td>
<td>HRP Goat Anti-Mouse Ig</td>
<td>1.0 ml</td>
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<tr>
<td>554655</td>
<td>Fixation Buffer</td>
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<td>558050</td>
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<tr>
<td>554723</td>
<td>Perm/Wash Buffer</td>
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<tr>
<td>554656</td>
<td>Stain Buffer (FBS)</td>
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<tr>
<td>353219</td>
<td>BD Falcon™ 96-well Imaging Plate</td>
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Product Notices

1. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.

2. Triton is a trademark of the Dow Chemical Company.

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


References


