Anti-Phospho-Histone H2A.X (S139) Antibody [SR33-09] - BSA and Azide free

HA750034

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat, Cynomolgus monkey
Applications:	WB, IHC-P, IF-Cell, IF-Tissue
Molecular Wt:	Predicted band size: 15 kDa
Clone number:	SR33-09

Description: Histone H2A.X is a member of the Histone H2A family, which is involved in nucleosomal organization of chromatin. An important phosphorylated form is γH2AX (S139), which forms when double-strand breaks appear. In humans and other eukaryotes, the DNA is wrapped around histone octamers, consisting of core histones H2A, H2B, H3 and H4, to form chromatin. H2AX contributes to nucleosome-formation, chromatin-remodeling and DNA repair, and is also used in vitro as an assay for double-strand breaks in dsDNA.

Immunogen: Synthetic phospho-peptide corresponding to residues surrounding Ser139 of Human Histone H2AX.

Positive control: NIH/3T3 cell lysate, NIH/3T3 treated with 25μM Etoposide for 5 hours cell lysate, HeLa treated with 20μM Etoposide for 2 hours whole cell lysate, HeLa treated with UV for 2 hours whole cell lysate, C6 treated with 25μM Etoposide for 5 hours whole cell lysate, rat brain tissue, mouse testis tissue, mouse small intestine tissue, human colon carcinoma tissue, human colon cancer tissue, mouse breast tissue, mouse skin tissue, HeLa cells treated with 20μM etoposide for 2 hours.

Subcellular location: Nucleus, Chromosome

Database links: SwissProt: P16104 Human | P27661 Mouse
Unigene: 2850 Rat

Recommended Dilutions:

WB	1:2,000-1:5,000
IHC-P	1:500-1:5,000
IF-Cell	1:2,000-1:3,000
IF-Tissue	1:500

Storage Buffer: 1*PBS (pH7.4).

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

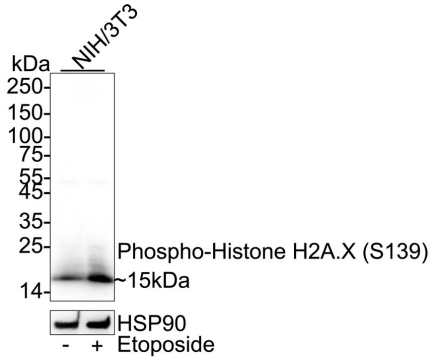

Fig1: Western blot analysis of Phospho-Histone H2A.X (S139) on different lysates with Rabbit anti-Phospho-Histone H2A.X (S139) antibody (HA750034) at 1/5,000 dilution.

Lane 1: NIH/3T3 cell lysate (20 µg/Lane)
Lane 2: NIH/3T3 treated with 25µM Etoposide for 5 hours cell lysate (20 µg/Lane)

Predicted band size: 15 kDa
Observed band size: 15 kDa

Exposure time: 6 seconds; ECL: K1801;
4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA750034) at 1/5,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

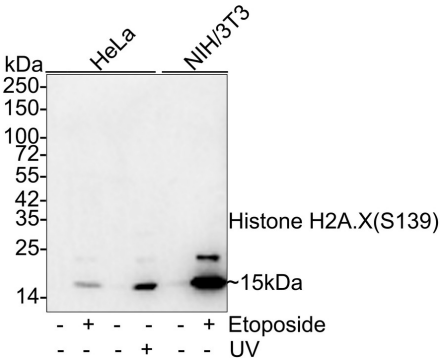

Fig2: Western blot analysis of Phospho-Histone H2A.X (S139) on different lysates with Rabbit anti-Phospho-Histone H2A.X (S139) antibody (HA750034) at 1/2,000 dilution.

Lane 1: HeLa whole cell lysate
Lane 2: HeLa treated with 20µM Etoposide for 2 hours whole cell lysate
Lane 3: HeLa whole cell lysate
Lane 4: HeLa treated with UV for 2 hours whole cell lysate
Lane 5: NIH/3T3 whole cell lysate
Lane 6: NIH/3T3 treated with 25µM Etoposide for 5 hours whole cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 15 kDa
Observed band size: 15/20 kDa

Exposure time: 53 seconds;
4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA750034) at 1/2,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/100,000 dilution was used for 1 hour at room temperature.

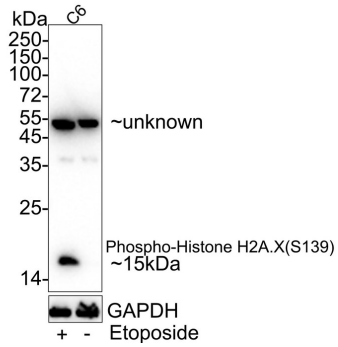

Fig3: Western blot analysis of Phospho-Histone H2A.X (S139) on different lysates with Rabbit anti-Phospho-Histone H2A.X (S139) antibody (HA750034) at 1/2,000 dilution.

Lane 1: C6 treated with 25μM Etoposide for 5 hours whole cell lysate
Lane 2: C6 whole cell lysate

Lysates/proteins at 20 μg/Lane.

Predicted band size: 15 kDa
Observed band size: 15 kDa

Exposure time: 20 seconds; ECL: K1801;
4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA750034) at 1/2,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

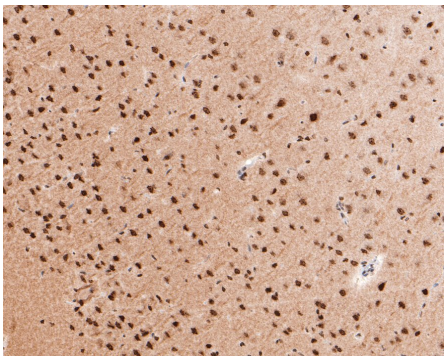

Fig4: Immunohistochemical analysis of paraffin-embedded rat brain tissue with Rabbit anti-Phospho-Histone H2A.X (S139) antibody (HA750034) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750034) at 1/500 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

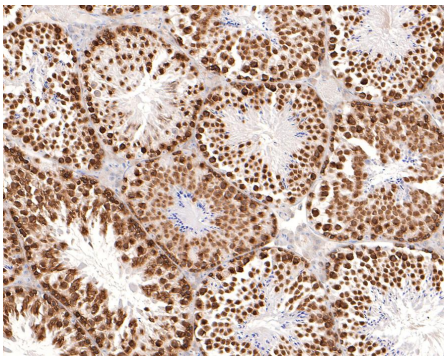

Fig5: Immunohistochemical analysis of paraffin-embedded mouse testis tissue with Rabbit anti-Phospho-Histone H2A.X (S139) antibody (HA750034) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750034) at 1/500 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

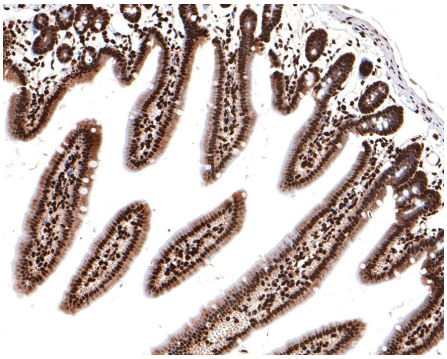

Fig6: Immunohistochemical analysis of paraffin-embedded mouse small intestine tissue with Rabbit anti-Phospho-Histone H2A.X (S139) antibody (HA750034) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750034) at 1/500 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

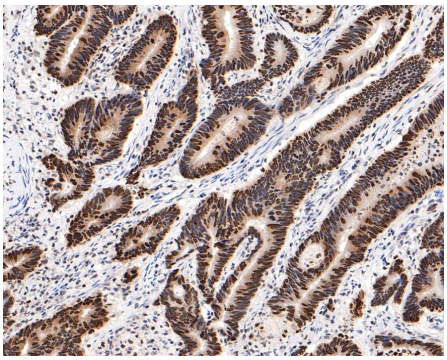

Fig7: Immunohistochemical analysis of paraffin-embedded human colon carcinoma tissue with Rabbit anti-Phospho-Histone H2A.X (S139) antibody (HA750034) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750034) at 1/500 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

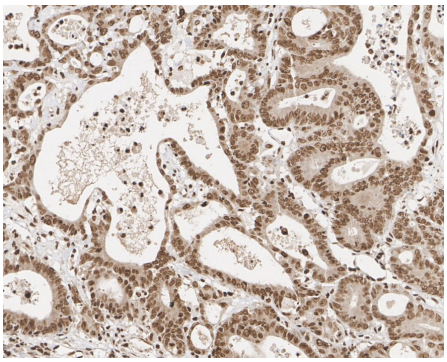

Fig8: Immunohistochemical analysis of paraffin-embedded human colon cancer tissue with Rabbit anti-Phospho-Histone H2A.X (S139) antibody (HA750034) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750034) at 1/500 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

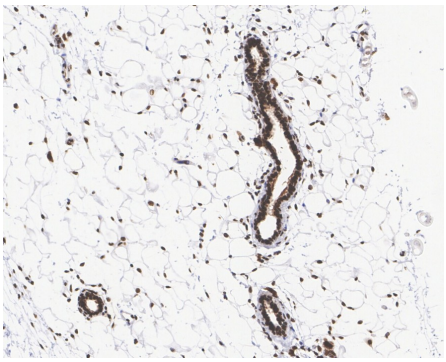

Fig9: Immunohistochemical analysis of paraffin-embedded mouse breast tissue with Rabbit anti-Phospho-Histone H2A.X (S139) antibody (HA750034) at 1/5,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750034) at 1/5,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

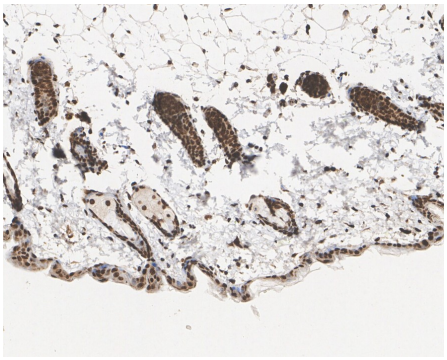

Fig10: Immunohistochemical analysis of paraffin-embedded mouse skin tissue with Rabbit anti-Phospho-Histone H2A.X (S139) antibody (HA750034) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750034) at 1/500 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

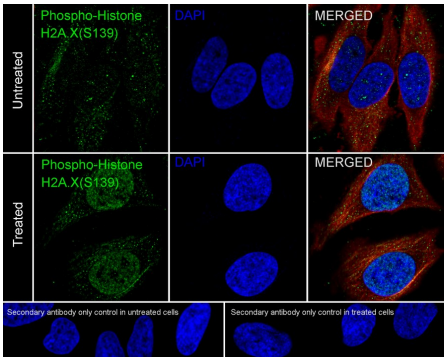

Fig11: Immunocytochemistry analysis of HeLa cells untreated / treated with 20μM etoposide for 2 hours labeling Phospho-Histone H2A.X (S139) with Rabbit anti-Phospho-Histone H2A.X (S139) antibody (HA750034) at 1/2,000 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-Phospho-Histone H2A.X (S139) antibody (HA750034) at 1/2,000 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (HA601187, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

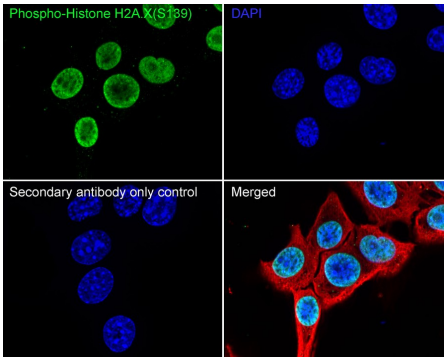

Fig12: Immunocytochemistry analysis of 4T1 cells labeling Phospho-Histone H2A.X (S139) with Rabbit anti-Phospho-Histone H2A.X (S139) antibody (HA750034) at 1/3,000 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-Phospho-Histone H2A.X (S139) antibody (HA750034) at 1/3,000 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (HA601187, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

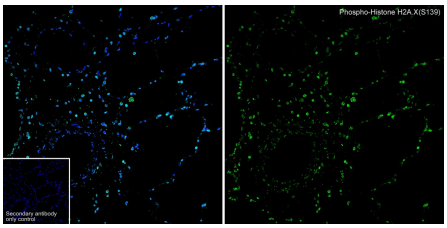

Fig13: Application: IF-tissue

Species: Cynomolgus monkey

Site: Lung

Sample: Paraffin-embedded section

Antibody concentration: 1/500

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

1. Kung, M.L. et al. 2015. Enhanced reactive oxygen species overexpression by CuO nanoparticles in poorly differentiated hepatocellular carcinoma cells. Nanoscale. 7: 1820-9.
2. Cilli, D. et al. 2014. Identification of the interactors of human nibrin (NBN) and of its 26 kDa and 70 kDa fragments arising from the NBN 657del5 founder mutation. PloS one. 9: e114651.