Anti-Histone H2A.x Antibody

ER1901-70

Product Type:	Rabbit polyclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, IF-Cell, IF-Tissue, FC
Molecular Wt:	15 kDa

Description: Histone H2A.X is a member of the Histone H2A family, which is involved in nucleosomal organization of chromatin. The H2AFX gene is located in close proximity to the Porphobilinogen deaminase (PBG-D) gene in both mouse and human, and maps to chromosome 9 and 11q23, respectively. H2A.X differs from the other members of the H2A family by the presence of a highly conserved C-terminal motif. It is rapidly phosphorylated in response to ionizing radiation and plays an important role in the recognition and repair of DNA double stranded breaks. The phosphorylated form of H2A.X, designated γ-H2A.X, forms nuclear foci at the heavy chain constant region of cells involved in class switch recombination (CSR), a region-specific DNA reaction that replaces one immunoglobulin heavy chain constant region gene with another. The phosphorylated γ-H2A.X is also thought to initiate subsequent repair factors, including Rad50, Rad51 and BRCA1.

Immunogen: Synthetic peptide within Human Histone H2AX aa 1-50 / 143.

Positive control: MCF-7 cell lysates, SHSY5Y, Rat testicular tissue, human lung cancer tissue, human liver tissue, mouse fallopian tube tissue.

Subcellular location: Nucleus

Database links: SwissProt: P16104 Human | P27661 Mouse

Recommended Dilutions:

WB	1:500-1:1000
IHC-P	1:100-1:500
IF-Cell	1:100-1:500
IF-Tissue	1:100-1:500
FC	1:50-1:100

Storage Buffer: 1*PBS (pH7.4), 0.2% BSA, 50% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.

Purity: Immunogen affinity purified.

Images

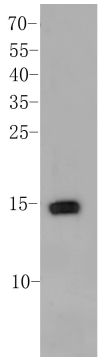

Fig1: Western blot analysis of Histone H2A.x on MCF-7 cell lysate. Proteins were transferred to a PVDF membrane and blocked with 5% BSA in PBS for 1 hour at room temperature. The primary antibody (ER1901-70, 1/500) was used in 5% BSA at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:5,000 dilution was used for 1 hour at room temperature.

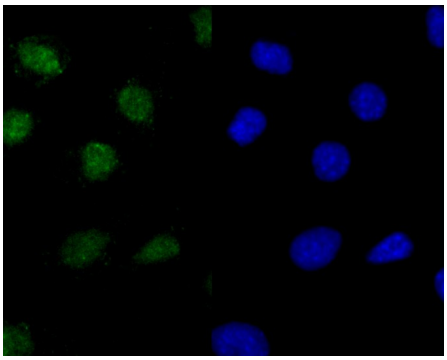

Fig2: ICC staining of Histone H2A.x in SHSY5Y cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ER1901-70, 1/100) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/100 dilution. The nuclear counter stain is DAPI (blue).

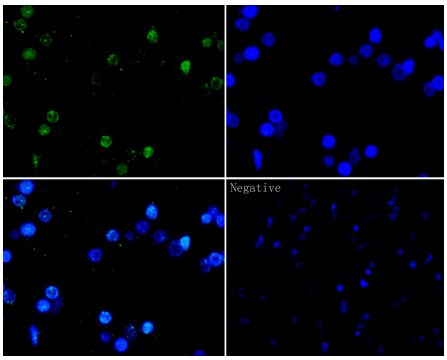

Fig3: Immunofluorescence analysis of paraffin-embedded rat brain using anti-Histone H2A.x antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature , washed with ddH_2O and PBS, and then probed with the primary antibody (ER1901-70, 1/100) at 4 ° C overnight. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/100 dilution. The nuclear counter stain is DAPI (blue)。

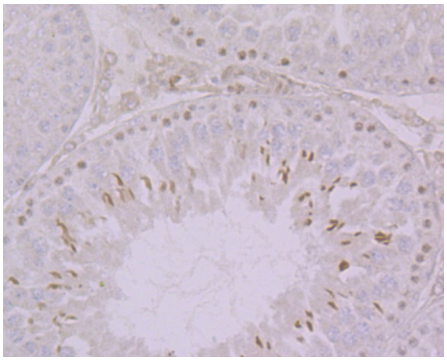

Fig4: Immunohistochemical analysis of paraffin-embedded Rat testicular tissue using anti-Histone H2A.x antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ER1901-70, 1/100) for 30 minutes 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.

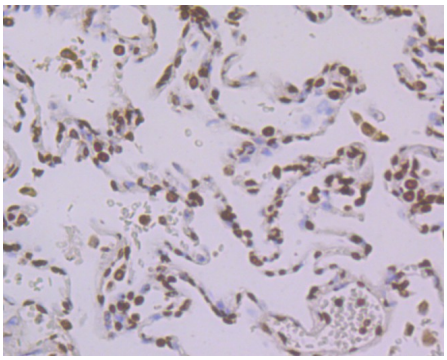

Fig5: Immunohistochemical analysis of paraffin-embedded human lung cancer tissue using anti-Histone H2A.x antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ER1901-70, 1/100) for 30 minutes 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.

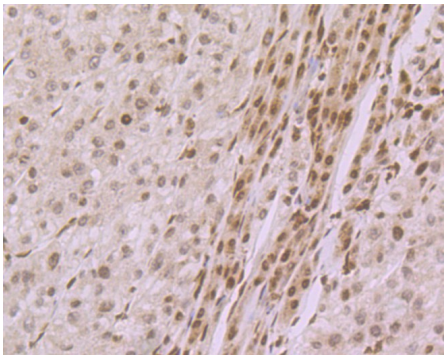

Fig6: Immunohistochemical analysis of paraffin-embedded human liver tissue using anti-Histone H2A.x antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ER1901-70, 1/100) for 30 minutes 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.

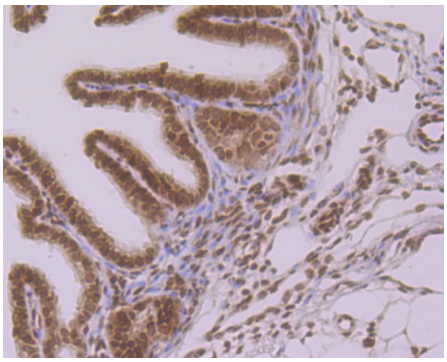

Fig7: Immunohistochemical analysis of paraffin-embedded mouse fallopian tube tissue using anti-Histone H2A.x antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ER1901-70, 1/100) for 30 minutes 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.

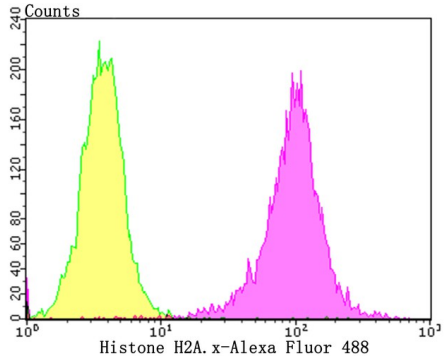

Fig8: Flow cytometric analysis of Histone H2A.x was done on SHSY5Y cells. The cells were fixed, permeabilized and stained with the primary antibody (ER1901-70, 1/100) (purple). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor 488-conjugated goat anti-rabbit IgG Secondary antibody at 1/500 dilution for 30 minutes.Unlabelled sample was used as a control (cells without incubation with primary antibody; yellow).

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

Background References

1. Paull T T et al. A critical role for histone H2AX in recruitment of repair factors to nuclear foci after DNA damage. Curr Biol 10:886-895 (2000).