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Rat Histone H2A.X Ready-To-Use IHC Kit

Cat.No: IHC0308R
Applications: **IHC-P**
Reactivity: Rat
Size: 50T
Assay type: Immunohistochemistry
Sample type: FFPE tissue
General Information:

Number	Component	Size	Concentration	Storage
1	PBS Buffer (powder)	2 L×2	20x	RT
2	Antigen Retrieval Buffer	20 ml	100x	2-8°C
3	Endogenous Peroxidase Blocking Buffer	3 ml	RTU	2-8°C, protect from light
4	Blocking Buffer	3 ml	RTU	2-8°C
5	Primary Antibody (Rat Histone H2A.X Recombinant Rabbit mAb)	6 ml	RTU	2-8°C
6	Secondary Antibody (Goat Anti-Rabbit IgG H&L, HRP conjugated)	6 ml	RTU	2-8°C
7	Chromogen Component A	0.3 ml	RTU	-20°C,protect from light
8	Chromogen Component B	0.3 ml	RTU	-20°C
9	Counter Staining Reagent	5 ml	RTU	RT
10	Mounting Media	5 ml	RTU	RT
11	Control slide (Rat colon)	1 slide	RTU	RT
12	Datasheet	1 copy		

Storage and Stability: Please store components at the temperatures indicated on the individual tube labels. The kit is stable for 6 months from the date of receipt.

Immunohistochemistry Protocol:

1. **Deparaffinization And Rehydration**

Immerse slides in fresh xylene for 15 minutes and then repeat two more times using separate containers. Immerse slides sequentially in 100%, 95%, 90%, 80%, and 70% ethanol solutions for 5 minutes each. Rinse slides 3 times with distilled water for 5 minutes each.

2. **Antigen Retrieval**

Add 100× **Antigen Retrieval Buffer** into distilled water to prepare a 1×solution. Boil slides in 1×solution at 95°C-100°C for 15 minutes. Move the slides to 1×solution at room temperature (RT) and allow them to stand for 20 minutes. Rinse 3 times with **PBS Buffer** (dissolve the powder in 2L distilled water) for 5 minutes each.

3. **Block Endogenous Peroxidase**

Drain the liquid off the slides and then use a hydrophobic IHC pen to draw circles on the slides around tissue sections. Add 2-4 drops of **Endogenous Peroxidase Blocking Buffer** directly on slides, covering the whole tissue and block slides for 15 minutes at RT. Rinse 3 times with **PBS Buffer** for 5 minutes each.

4. **Serum Blocking**

Block with 2-4 drops of **Blocking Buffer** for 20 minutes at RT.

5. **Primary Antibody Incubation**

Drain blocking buffer from slides. Incubate slides with 2-4 drops of **Rat Histone H2A.X Recombinant Rabbit mAb** overnight at 4°C or 1-2 hours at RT. Rinse 3 times with **PBS Buffer** for 5 minutes each.

6. **Secondary Antibody Incubation**

Incubate slides with 2-4 drops of **Goat Anti-Rabbit IgG H&L, HRP conjugated** for 1-2 hours at RT. Rinse slides 3 times with **PBS Buffer** for 5 minutes each.

7. **Signal Development**

Remove residual liquid around the tissue section. Add 50ul fresh **DAB Buffer** (**Chromogen Component A : Chromogen Component B : PBS Buffer=1:1:18**) to cover the tissue. Monitor the reaction under the microscope until a brown color is visible (approximate 3-5 minutes at RT). Stop reaction immediately by rinsing with distilled water. Rinse slides 3 times with distilled water for 5 minutes each.

8. **Counterstain**

Counterstain with an appropriate amount of **Counter Staining Reagent** for 3-5 minutes at RT. Rinse slides with distilled water for 5 minutes. Use 2-4 drops of **Differentiation reagent** to cover the tissue for 30 seconds. Rinse slides twice with distilled water for 5 minutes each.

9. **Dehydration Sheet**

Immerse slides sequentially in 70%, 80%, 90%, 95%, and 100% ethanol for 5 minutes each at RT. Immerse slides in 2 changes of fresh xylene, 15 minutes each. Drop some **Mounting Media** on the tissue. Mount coverslips.

Notes:

1. The positive control slide provided in the kit allows you to be sure that the experimental set-up is working properly.
2. Do not allow slides to dry at any time during this procedure.

3. Please don't replace the matching reagents in this product with other manufacturers' products.

4. As DAB is a carcinogen, please take necessary precautions.

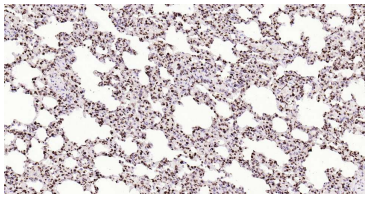
5. PBS (reagent 1) can be stored for one week at 4°C after preparation; The antigen retrieval buffer (1×reagent 2) and the chromogenic agent (the mixture of reagents 7 and 8) should be prepared right before each assay.

Please cite this product as " IHC0308R, Bioss Antibodies". Citation example: " Rat Tissue sections using H2AX IHC Kit (IHC0308R, Bioss Antibodies) were stained for H2AX according to the manufacturer's instructions."

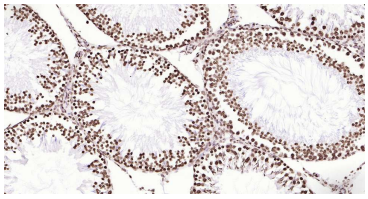
Introduction:

Histone H2A.X (H2AX) is a member of the histone H2A family which is one of the four core histones making up the nucleosome core particle. In eukaryotes, DNA double strand breaks (DSBs) have been shown to trigger the phosphorylation of serine 139 at the carboxy terminus of histone H2AX resulting in gamma-H2AX. The phosphorylation of H2AX can be detected by Western blotting or immunofluorescence, revealing the frequency of DSBs. The phosphatidylinositol 3-kinases have been implicated in H2AX phosphorylation, but it is unclear if ATM is the primary H2AX kinase or if other members of the family such as DNA-PK and ATR contribute in a similar manner. Structurally, H2A.x contains 143 amino acid residues. Histone H2A.X is considered a basal histone, being synthesized in G1 as well as in S-phase, and its mRNA contains polyA addition motifs and a polyA tail along with the conserved stem-loop and U7 binding sequences involved in the processing and stability of replication type histone mRNAs. There are two forms of Histone H2A.X mRNA, one about 1600 bases long and contains polyA; the other about 575 bases long, lacking polyA. The short form behaves as a replication type histone mRNA, while the longer behaves as a basal type histone mRNA. Histone H2A.X maps to the 11q23.2-q23.3 region of the human chromosome. Histone H2A.x contributes to histone-formation and therefore the structure of DNA. Histone H2A variant H2A.x specifically regulates the interaction of MDC1 (mediator of DNA damage checkpoint protein 1), a DNA repair protein to the sites of DNA damage.

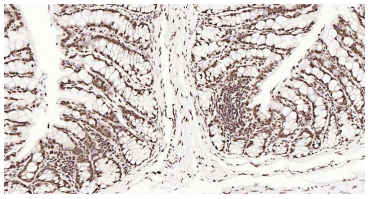
Validation Data



Immunohistochemical analysis of paraffin embedded rat lung tissue slide using IHC0308R (Rat Histone H2A.X Kit).



Immunohistochemical analysis of paraffin embedded rat testis tissue slide using IHC0308R (Rat Histone H2A.X Kit).



Immunohistochemical analysis of paraffin embedded rat colon tissue slide using IHC0308R (Rat Histone H2A.X Kit).