Anti-Histone H3 Antibody [6-A7-R] - BSA and Azide free HA610203

Product Type:	Recombinant Mouse monoclonal IgG1, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, IF-Tissue, ChIP
Molecular Wt:	Predicted band size: 15 kDa
Clone number:	6-A7-R
Description:	The nucleosome, made up of DNA wound around eight core histone proteins (two each of H2A, H2B, H3, and H4), is the primary building block of chromatin. Histones thereby play a central role in transcription regulation, DNA repair, DNA replication and chromosomal stability. DNA accessibility is regulated via a complex set of post-translational modifications of histones, also called histone code, and nucleosome remodeling.
lmmunogen:	Synthetic peptide within Human Histone H3 aa 1-50 / 136.
Positive control:	HeLa cell lysate, A549 cell lysate, NIH/3T3 cell lysate, PC-12 cell lysate, C6 cell lysate, rat testis tissue lysate, mouse skin tissue lysate, human liver tissue, mouse epididymis tissue, mouse testis tissue, rat brain tissue.
Subcellular location:	Nucleus.
Database links:	SwissProt: P68431 human P84243 human Q16695 human Q6NXT2 human Q71DI3 human P68433 mouse P84228 mouse Q6LED0 rat
Recommended Dilutions: WB IHC-P IF-Tissue ChIP	1:5,000 1:1,000 1:200 Use 0.5~2 μg for 25 μg of chromatin.
Storage Buffer:	PBS (pH7.4).
Storage Instruction:	Store at +4 $^\circ\!\!\mathbb{C}$ after thawing. Aliquot store at -20 $^\circ\!\!\mathbb{C}$. Avoid repeated freeze / thaw cycles.

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Orders:0086-571-88062880

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Images

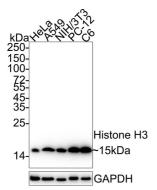


Fig1: Western blot analysis of Histone H3 on different lysates with Mouse anti-Histone H3 antibody (HA610203) at 1/5,000 dilution.

Lane 1: HeLa cell lysate Lane 2: A549 cell lysate Lane 3: NIH/3T3 cell lysate Lane 4: PC-12 cell lysate Lane 5: C6 cell lysate

Lysates/proteins at 20 µg/Lane.

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

Exposure time: 14 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 (HA610203) at 1/5,000 dilution was used in 5% NFDM/TBST at 4° C overnight. Anti-Mouse IgG for IP Nanosecondary antibody (NBI02H) at 1/5,000 dilution was used for 1 hour at room temperature.

Fig2: Western blot analysis of Histone H3 on different lysates with Mouse anti-Histone H3 antibody (HA610203) at 1/5,000 dilution.

Lane 1: Rat testis tissue lysate Lane 2: Mouse skin tissue lysate

Lysates/proteins at 20 µg/Lane.

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

Exposure time: 15 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 (HA610203) at 1/5,000 dilution was used in 5% NFDM/TBST at 4° C overnight. Anti-Mouse IgG for IP Nanosecondary antibody (NBI02H) at 1/5,000 dilution was used for 1 hour at room temperature.

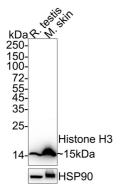
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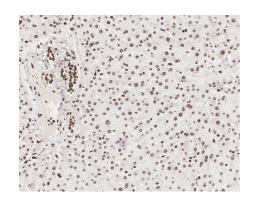


Fig3: Immunohistochemical analysis of paraffin-embedded human liver tissue with Mouse anti-Histone H3 antibody (HA610203) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (HA610203) at 1/1,000 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.

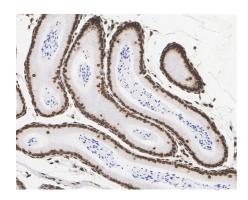


Fig4: Immunohistochemical analysis of paraffin-embedded mouse epididymis tissue with Mouse anti-Histone H3 antibody (HA610203) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (HA610203) at 1/1,000 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.

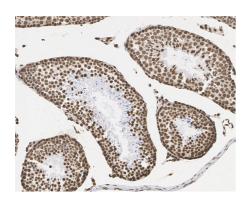


Fig5: Immunohistochemical analysis of paraffin-embedded mouse testis tissue with Mouse anti-Histone H3 antibody (HA610203) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (HA610203) at 1/1,000 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.

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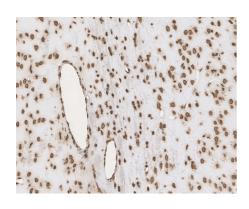


Fig6: Immunohistochemical analysis of paraffin-embedded rat brain tissue with Mouse anti-Histone H3 antibody (HA610203) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (HA610203) at 1/1,000 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.

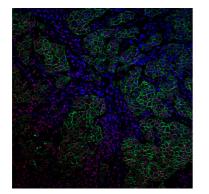


Fig7: Immunofluorescence analysis of paraffin-embedded human breast cancer tissue labeling Histone H3 (HA610203, red) and HER2 / ErbB2 (HA721210, green).

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS. And then probed with the primary antibodies Histone H3 (HA610203, red) at 1/200 dilution and HER2 / ErbB2 (HA721210, green) at 1/200 dilution overnight at 4 $^{\circ}$ C, washed with PBS.

iFluor[™] 594 conjugate-Goat anti-Mouse IgG (HA1126) and iFluor[™] 488 conjugate-Goat anti-Rabbit IgG (HA1121) were used as the secondary antibodies at 1/1,000 dilution. DAPI was used as nuclear counterstain.

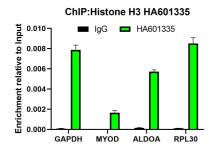


Fig8: Chromatin immunoprecipitations were performed with crosslinked chromatin from HeLa cells with Histone H3 (HA610203) or Normal Mouse IgG according to the ChIP protocol. The enriched DNA was quantified by real-time PCR using indicated primers. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin, which is equivalent to one.

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

Background References

- 1. Flanagan J.F., Mi L.-Z., Chruszcz M., Cymborowski M., Clines K.L., Kim Y., Minor W., Rastinejad F., Khorasanizadeh S."Double chromodomains cooperate to recognize the methylated histone H3 tail."Nature 438:1181-1185(2005)
- "Arginine methylation of the histone H3 tail impedes effector binding."Iberg A.N., Espejo A., Cheng D., Kim D., Michaud-Levesque J., Richard S., Bedford M.T.J. Biol. Chem. 283:3006-3010(2008)

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Applications:WB=Western blot IHC-P=Immunohistochemistry (paraffin) IF-Cell=Immunofluorescence (Cell) IF-Tissue=Immunofluorescence (Tissue) FC=Flow cytometry IP=Immunoprecipitation

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