Anti-Histone H3 Antibody [JJ090-07]

ET1701-64

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, ChIP, IP
Molecular Wt:	Predicted band size: 15 kDa
Clone number:	JJ090-07

Description: In eukaryotes, DNA is wrapped around histone octamers to form the basic unit of chromatin structure. The octamer is composed of histones H2A, H2B, H3 and H4, and it associates with approximately 200 base pairs of DNA to form the nucleosome. The association of DNA with histones results in dense packing of chromatin, which restricts proteins involved in gene transcription from binding to DNA. p300 preferentially acetylates Histone H3 at lysines 14 and 18 and Histone H4 at lysines 5 and 8. PCAF in its native form, primarily acetylates Histone H3 at lysine 14 to a monoacetylated form, and less efficiently acetylates Histone H4 at lysine 8. Histone H4 may also be acetylated at lysines 12 and 16, and the involvement of acetylated H4 with Histones H2A, H2B and H3 suggests that acetylated histones may be involved in dynamic chromatin remodeling.

Immunogen: Recombinant protein within human Histone H3 aa 85-136/136.

Positive control: HeLa cell lysate, A549 cell lysate, HT-29 cell lysate, HEK-293 cell lysate, C2C12 cell lysate, L-929 cell lysate, C6 cell lysate, HeLa, human kidney tissue, human skin tissue, mouse liver tissue, mouse kidney tissue, rat liver tissue, rat kidney tissue, rat skin tissue, human liver tissue, mouse testis tissue, rat testis tissue.

Subcellular location: Nucleus, Chromosome.

Database links: SwissProt: P68431 Human | P84243 Human | Q16695 Human | Q6NXT2 Human | Q71DI3 Human | P68433 Mouse | P84228 Mouse | Q6LED0 Rat

Recommended Dilutions:

WB	1:20,000
IF-Cell	1:100
IF-Tissue	1:100
IHC-P	1:5,000-1:10,000
ChIP	Use 0.5~2 μg for 25 μg of chromatin.
IP	1-2μg/sample

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

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

Purity: Protein A affinity purified.

Images

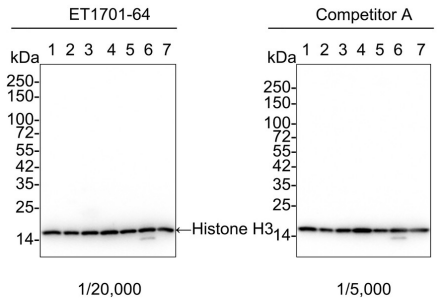

Fig1: Western blot analysis of Histone H3 on different lysates with Rabbit anti-Histone H3 antibody (ET1701-64) at 1/20,000 dilution and competitor's antibody at 1/5,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: A549 cell lysate
Lane 3: HT-29 cell lysate
Lane 4: HEK-293 cell lysate
Lane 5: C2C12 cell lysate
Lane 6: L-929 cell lysate
Lane 7: C6 cell lysate

Lysates/proteins at 10 µg/Lane.

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

Exposure time: 18 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 (ET1701-64) at 1/20,000 dilution and competitor's antibody at 1/5,000 dilution were 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.

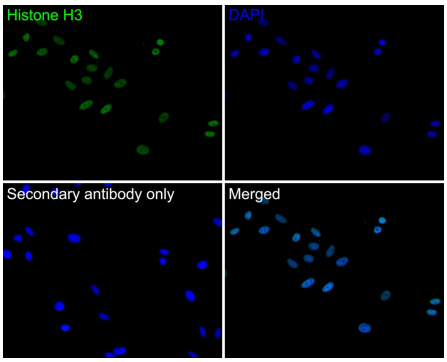

Fig2: Immunocytochemistry analysis of HeLa cells labeling Histone H3 with Rabbit anti-Histone H3 antibody (ET1701-64) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 ℃, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-Histone H3 antibody (ET1701-64) at 1/100 dilution in 2% negative goat serum 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.

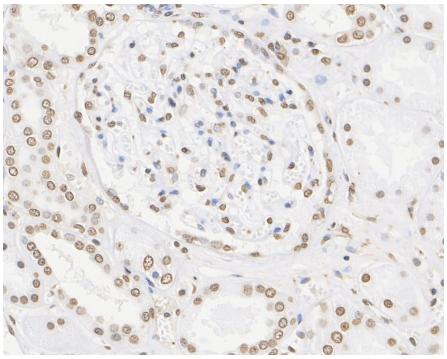

Fig3: Immunohistochemical analysis of paraffin-embedded human kidney tissue with Rabbit anti-Histone H3 antibody (ET1701-64) at 1/10,000 dilution.

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 ddH_2O and PBS, and then probed with the primary antibody (ET1701-64) at 1/10,000 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.

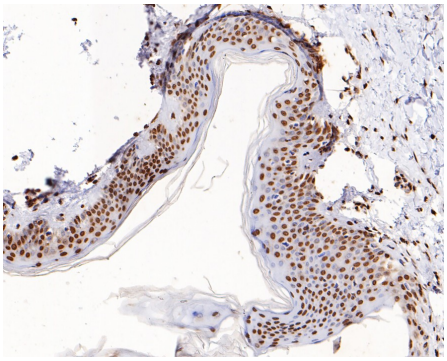

Fig4: Immunohistochemical analysis of paraffin-embedded human skin tissue with Rabbit anti-Histone H3 antibody (ET1701-64) at 1/5,000 dilution.

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 ddH_2O and PBS, and then probed with the primary antibody (ET1701-64) 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.

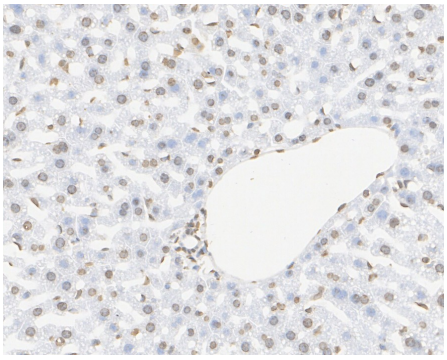

Fig5: Immunohistochemical analysis of paraffin-embedded mouse liver tissue with Rabbit anti-Histone H3 antibody (ET1701-64) at 1/10,000 dilution.

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 ddH_2O and PBS, and then probed with the primary antibody (ET1701-64) at 1/10,000 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.

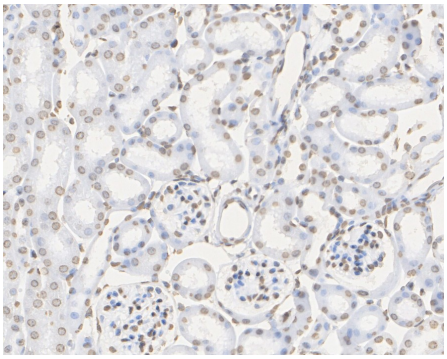

Fig6: Immunohistochemical analysis of paraffin-embedded mouse kidney tissue with Rabbit anti-Histone H3 antibody (ET1701-64) at 1/10,000 dilution.

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 ddH_2O and PBS, and then probed with the primary antibody (ET1701-64) at 1/10,000 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.

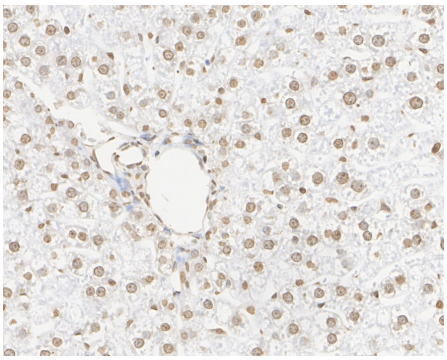

Fig7: Immunohistochemical analysis of paraffin-embedded rat liver tissue with Rabbit anti-Histone H3 antibody (ET1701-64) at 1/10,000 dilution.

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 ddH_2O and PBS, and then probed with the primary antibody (ET1701-64) at 1/10,000 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.

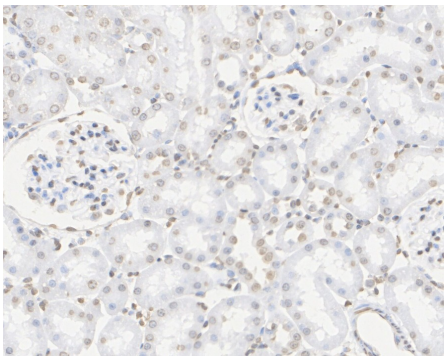

Fig8: Immunohistochemical analysis of paraffin-embedded rat kidney tissue with Rabbit anti-Histone H3 antibody (ET1701-64) at 1/10,000 dilution.

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 ddH_2O and PBS, and then probed with the primary antibody (ET1701-64) at 1/10,000 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.

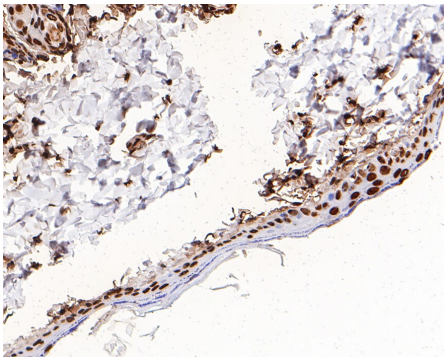

Fig9: Immunohistochemical analysis of paraffin-embedded rat skin tissue with Rabbit anti-Histone H3 antibody (ET1701-64) at 1/5,000 dilution.

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 ddH_2O and PBS, and then probed with the primary antibody (ET1701-64) 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.

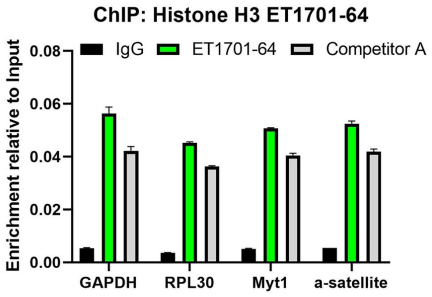

Fig10: Chromatin immunoprecipitations were performed with cross-linked chromatin from HeLa cells and Histone H3 (ET1701-64) / Competitor's antibody / Normal Rabbit IgG according to the ChIP protocol. The enriched DNA was quantified by real-time PCR using indicated primers. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin, which is equivalent to one.

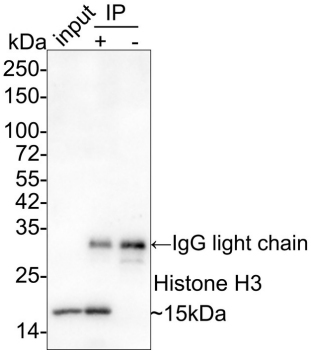

Fig11: Histone H3 was immunoprecipitated in 0.2mg HeLa cell lysate with ET1701-64 at 2 µg/25 µl agarose. Western blot was performed from the immunoprecipitate using ET1701-64 at 1/20,000 dilution. Anti-Rabbit IgG for IP Nano-secondary antibody (NBI01H) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: HeLa cell lysate (input)
Lane 2: ET1701-64 IP in HeLa cell lysate
Lane 3: Rabbit IgG instead of ET1701-64 in HeLa cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 1 minute 59 seconds

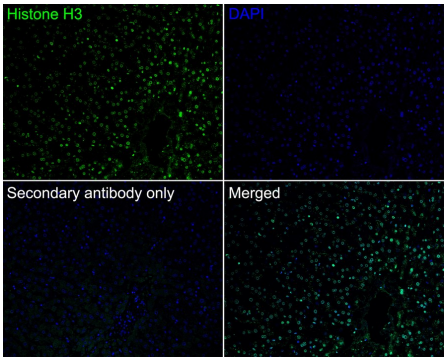

Fig12: Immunofluorescence analysis of paraffin-embedded human liver tissue labeling Histone H3 with Rabbit anti-Histone H3 antibody (ET1701-64) at 1/100 dilution.

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 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (ET1701-64, green) at 1/100 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

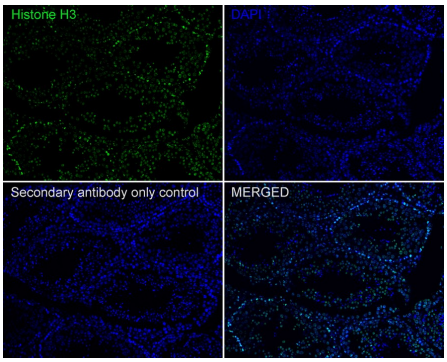

Fig13: Immunofluorescence analysis of paraffin-embedded mouse testis tissue labeling Histone H3 with Rabbit anti-Histone H3 antibody (ET1701-64) at 1/100 dilution.

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 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (ET1701-64, green) at 1/100 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

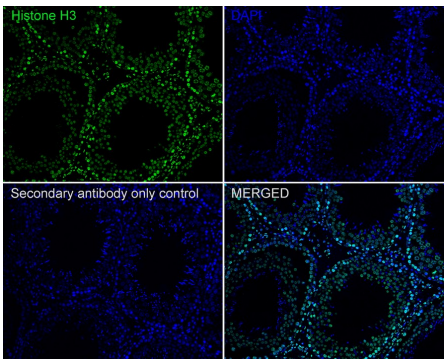

Fig14: Immunofluorescence analysis of paraffin-embedded rat testis tissue labeling Histone H3 with Rabbit anti-Histone H3 antibody (ET1701-64) at 1/100 dilution.

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 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (ET1701-64, green) at 1/100 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

Background References

1. Wani S et al. Human SCP4 is a chromatin-associated CTD phosphatase and exhibits the dynamic translocation during erythroid differentiation. J Biochem 160:111-20 (2016).
2. Ni JZ et al. A transgenerational role of the germline nuclear RNAi pathway in repressing heat stress-induced transcriptional activation in C. elegans. Epigenetics Chromatin 9:3 (2016).