Anti-Histone H3 (tri methyl K9) Antibody [1-6]

M1112-3

Product Type:	Mouse monoclonal IgG1, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IHC-P, IF-Tissue
Molecular Wt:	Predicted band size: 15 kDa
Clone number:	1-6

Description: Eukaryotic histones are basic and water soluble nuclear proteins that form hetero-octameric nucleosome particles by wrapping 146 base pairs of DNA in a left-handed super-helical turn sequentially to form chromosomal fibers. Two molecules of each of the four core histones (H2A, H2B, H3 and H4) form the octamer, which is comprised of two H2A-H2B dimers and two H3-H4 dimers, forming two nearly symmetrical halves by tertiary structure. Histones are subject to posttranslational modification by enzymes primarily on their N-terminal tails, but also in their globular domains. Human Histone H3 is subject to trimethylation at Lys 9, a modification that may be necessary for select DNA transactions or chromatin state transitions. Acetylation is generally linked to gene activation. Acetylation on Lys-10 (H3K9ac) impairs methylation at Arg-9 (H3R8me2s). Acetylation on Lys-19 (H3K18ac) and Lys-24 (H3K24ac) favors methylation at Arg-18 (H3R17me). Citrullination at Arg-9 (H3R8ci) and/or Arg-18 (H3R17ci) by PADI4 impairs methylation and represses transcription. Asymmetric dimethylation at Arg-18 (H3R17me2a) by CARM1 is linked to gene activation. Symmetric dimethylation at Arg-9 (H3R8me2s) by PRMT5 is linked to gene repression. Asymmetric dimethylation at Arg-3 (H3R2me2a) by PRMT6 is linked to gene repression and is mutually exclusive with H3 Lys-5 methylation (H3K4me2 and H3K4me3).

Immunogen: Synthetic peptide within N terminal human Histone H3 including tri methyl K9 conjugated to keyhole limpet haemocyanin.

Positive control: F9 cell lysate, PC-12 cell lysate, NCCIT cell lysate, HeLa, NIH/3T3, human testis tissue, mouse testis tissue, mouse epididymis tissue, rat testis tissue.

Subcellular location: Nucleus.

Database links: SwissProt: P68431 Human | P68433 Mouse | Q6LED0 Rat

Recommended Dilutions:

WB	1:5,000-1:10,000
IF-Cell	1:250
IHC-P	1:1,000
IF-Tissue	1:200

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: Protein G affinity purified.

Images

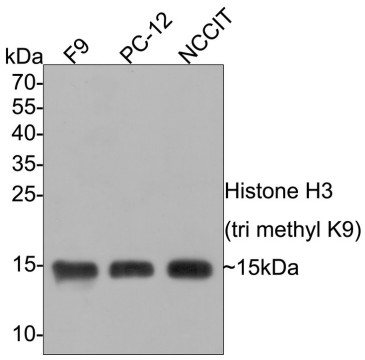

Fig1: Western blot analysis of Histone H3 (tri methyl K9) on different lysates with Mouse anti-Histone H3 (tri methyl K9) antibody (M1112-3) at 1/1,000 dilution.

Lane 1: F9 cell lysate
Lane 2: PC-12 cell lysate
Lane 3: NCCIT cell lysate

Lysates/proteins at 10 µg/Lane.

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

Exposure time: minute;

15% 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 (M1112-3) at 1/1,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1:100,000 dilution was used for 1 hour at room temperature.

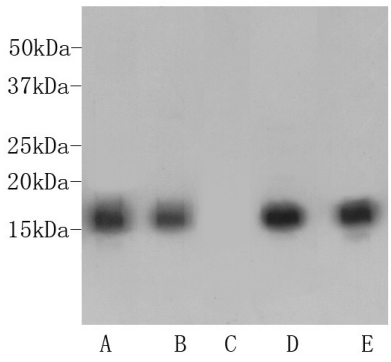

Fig2: Western blot analysis of Tri-Methyl-Histone H3 (Lys9) on different lysates. Proteins were transferred to a PVDF membrane and blocked with 5% BSA in PBS for 1 hour at room temperature. The primary antibody (M1112-3, 1/2,000) was used in 5% BSA at room temperature for 2 hours. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1:5,000 dilution was used for 1 hour at room temperature.

Positive control:

Lane A: F9 cell lysate
Lane B: F9+non-methyl peptide
Lane C: F9+Tri-methl (lys9) peptide
Lane D: PC12 cell lysate
Lane E: NCCIT cell lysate

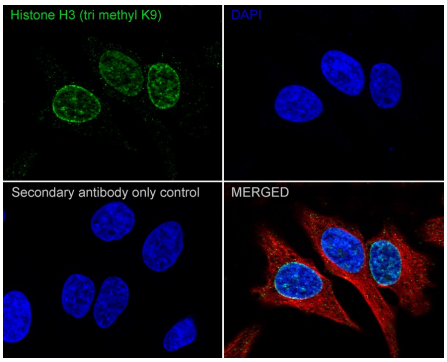

Fig3: Immunocytochemistry analysis of HeLa cells labeling Histone H3 (tri methyl K9) with Mouse anti-Histone H3 (tri methyl K9) antibody (M1112-3) at 1/250 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 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 Mouse anti-Histone H3 (tri methyl K9) antibody (M1112-3) at 1/250 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Mouse IgG H&L (iFluor™ 488, HA1125) 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 (ET1602-4, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Rabbit IgG H&L (iFluor™ 594, HA1122) were used as the secondary antibody at 1/1,000 dilution.

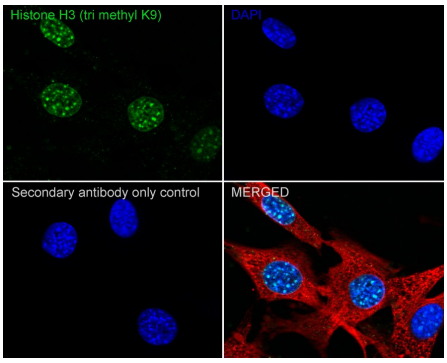

Fig4: Immunocytochemistry analysis of NIH/3T3 cells labeling Histone H3 (tri methyl K9) with Mouse anti-Histone H3 (tri methyl K9) antibody (M1112-3) at 1/250 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 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 Mouse anti-Histone H3 (tri methyl K9) antibody (M1112-3) at 1/250 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Mouse IgG H&L (iFluor™ 488, HA1125) 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.

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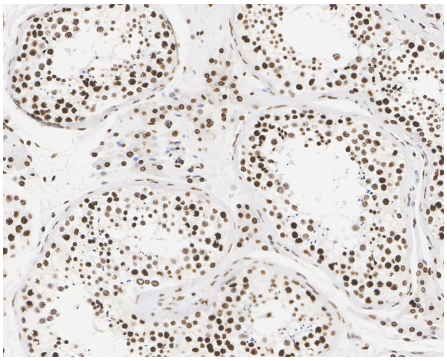

Fig5: Immunohistochemical analysis of paraffin-embedded human testis tissue with Mouse anti-Histone H3 (tri methyl K9) antibody (M1112-3) at 1/1,000 dilution.

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 ddH_2O and PBS, and then probed with the primary antibody (M1112-3) 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.

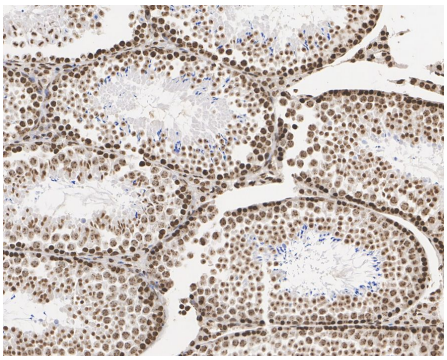

Fig6: Immunohistochemical analysis of paraffin-embedded mouse testis tissue with Mouse anti-Histone H3 (tri methyl K9) antibody (M1112-3) at 1/1,000 dilution.

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 ddH_2O and PBS, and then probed with the primary antibody (M1112-3) 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.

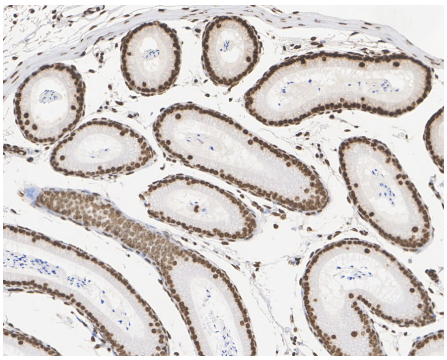

Fig7: Immunohistochemical analysis of paraffin-embedded mouse epididymis tissue with Mouse anti-Histone H3 (tri methyl K9) antibody (M1112-3) at 1/1,000 dilution.

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 ddH_2O and PBS, and then probed with the primary antibody (M1112-3) 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.

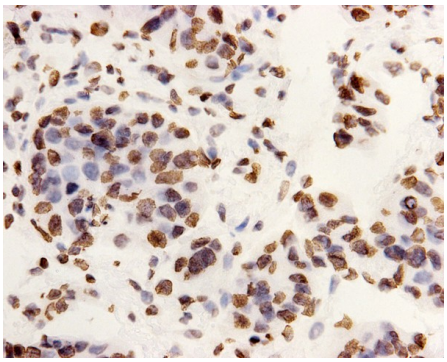

Fig8: Immunohistochemical analysis of paraffin-embedded rat testis tissue using anti-Tri-Methyl-Histone H3 (Lys9) antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (M1112-3, 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.

Background References

1. Chang, Q. et al. 2008. Sustained JNK1 activation is associated with altered Histone H3 methylations in human liver cancer. J. Hepatol. E-published.
2. Jin, Y. et al. 2008. Genetic and genome-wide analysis of simultaneous mutations in acetylated and methylated lysine residues in Histone H3 in Saccharomyces cerevisiae. Genetics E-published.