Anti-Histone H3 (di methyl K4) Antibody

R1110-3

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

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. Methylation at Lys-5 (H3K4me), Lys-37 (H3K36me) and Lys-80 (H3K79me) are linked to gene activation. Methylation at Lys-5 (H3K4me) facilitates subsequent acetylation of H3 and H4. Methylation at Lys-80 (H3K79me) is associated with DNA double-strand break (DSB) responses and is a specific target for TP53BP1. Methylation at Lys-10 (H3K9me) and Lys-28 (H3K27me) are linked to gene repression. Methylation at Lys-10 (H3K9me) is a specific target for HP1 proteins (CBX1, CBX3 and CBX5) and prevents subsequent phosphorylation at Ser-11 (H3S10ph) and acetylation of H3 and H4. Methylation at Lys-5 (H3K4me) and Lys-80 (H3K79me) require preliminary monoubiquitination of H2B at 'Lys-120'. Methylation at Lys-10 (H3K9me) and Lys-28 (H3K27me) are enriched in inactive X chromosome chromatin.

Immunogen: Synthetic peptide of the N terminal residues of Human Di-mehtyl-Histone H3(Lys4).

Positive control: Human liver tissue, Histone, F9, F9 treated with Histone H3 peptide-unmodifed, F9 treated with Histone H3 peptide-di-methyl K4, Ags, Hela, HepG2, rat testis tissue, mouse testis tissue.

Subcellular location: Nucleus.

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

Recommended Dilutions:

WB	1:1,000
IF-Cell	1:50-1:200
IHC-P	1:50-1:200
FC	1:50-1:100
Dot Blot	1:1,000

Storage Buffer: 1*PBS (pH7.4), 0.2% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

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

Purity: Immunogen affinity purified.

Images

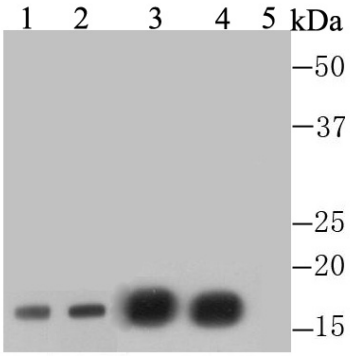

Fig1: Western blot analysis of Histone H3 (di methyl K4) on different lysates. Proteins were transferred to a PVDF membrane and blocked with 5% BSA in PBS for 1 hour at room temperature. The primary antibody was used at a 1:1,000 dilution in 5% BSA at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:5,000 dilution was used for 1 hour at room temperature.

Positive control:

Lane 1: Human liver tissue lysate, untreated
Lane 2: Histone lysate, purified from 293T
Lane 3: F9 cell lysate, untreated
Lane 4: F9 cell lysate, treated with Histone H3 peptide-unmodifed
Lane 5: F9 cell lysate, treated with Histone H3 peptide-di-methyl K4

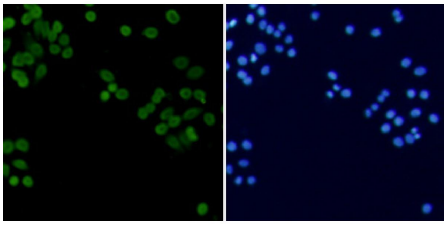

Fig2: ICC staining Histone H3 (di methyl K4) in Ags cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the antibody (R1110-3) at a dilution of 1:200 for 1 hour at room temperature, washed with PBS. Alexa Fluorc™ 488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/100 dilution. The nuclear counter stain is DAPI (blue).

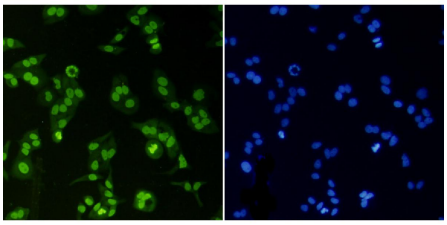

Fig3: ICC staining Histone H3 (di methyl K4) in Hela cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the antibody (R1110-3) at a dilution of 1:200 for 1 hour at room temperature, washed with PBS. Alexa Fluorc™ 488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/100 dilution. The nuclear counter stain is DAPI (blue).

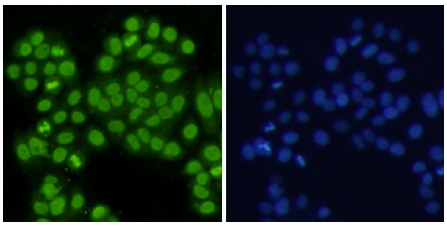

Fig4: ICC staining Histone H3 (di methyl K4) in HepG2 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the antibody (R1110-3) at a dilution of 1:200 for 1 hour at room temperature, washed with PBS. Alexa Fluorc™ 488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/100 dilution. The nuclear counter stain is DAPI (blue).

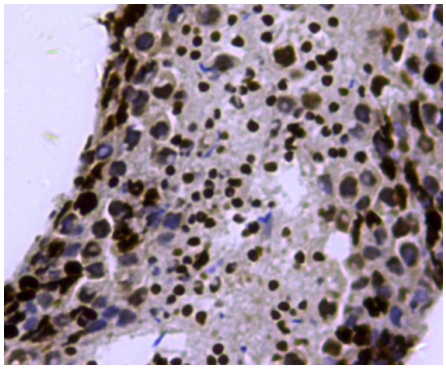

Fig5: Immunohistochemical analysis of paraffin-embedded rat testis tissue using anti-Histone H3 (di methyl K4) antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the antibody (R1110-3) at 1/200 dilution, for 30 minutes at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chrogen. Counter stained with hematoxylin and mounted with DPX.

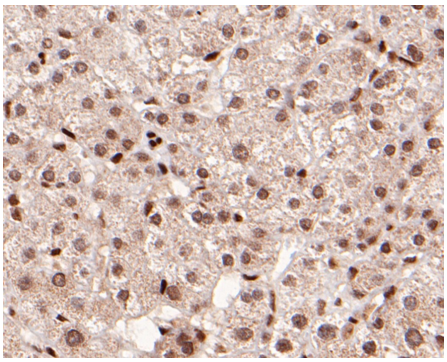

Fig6: Immunohistochemical analysis of paraffin-embedded human liver tissue with Rabbit anti-Histone H3 (di methyl K4) antibody (R1110-3) at 1/1,000 dilution.

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 (R1110-3) 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.

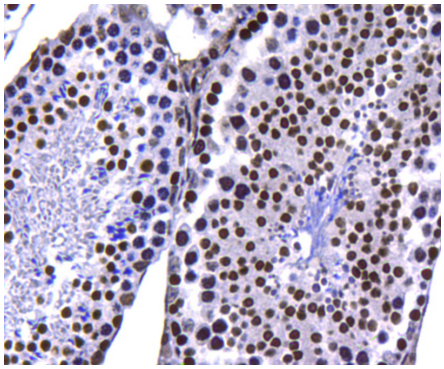

Fig7: Immunohistochemical analysis of paraffin-embedded mouse testis tissue using anti-Histone H3 (di methyl K4) antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the antibody (R1110-3) at 1/200 dilution, for 30 minutes at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chrogen. Counter stained with hematoxylin and mounted with DPX.

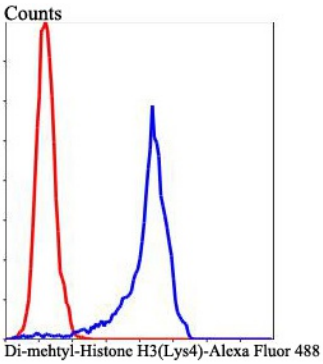

Fig8: Flow cytometric analysis of Histone H3 (di methyl K4) was done on Hela cells. The cells were fixed, permeabilized and stained with Histone H3 (di methyl K4) antibody at 1/100 dilution (blue) compared with an unlabelled control (cells without incubation with primary antibody; red). After incubation of the primary antibody on room temperature for an hour, the cells was stained with a Alexa Fluor™ 488-conjugated goat anti-rabbit IgG Secondary antibody at 1/500 dilution for 30 minutes.

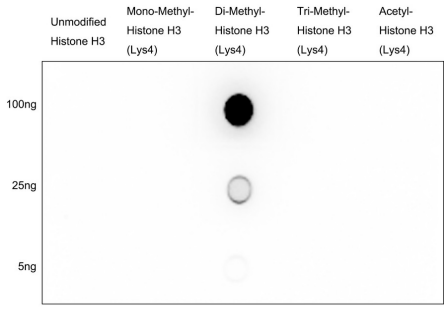

Fig9: Dot blot analysis of Histone H3 (di methyl K4) on different proteins with Rabbit anti-Histone H3 (di methyl K4) antibody (R1110-3) at 1/1,000 dilution. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution for 1 hour at room temperature.

Lane 1: Unmodified Histone H3 (negative)
Lane 2: Mono-Methyl-Histone H3 (Lys4) (negative)
Lane 3: Di-Methyl-Histone H3 (Lys4) (positive)
Lane 4: Tri-Methyl-Histone H3 (Lys4) (negative)
Lane 5: Acetyl-Histone H3 (Lys4) (negative)

Proteins loading: 100ng, 25ng, 5ng;

Blocking and dilution buffer: 5% NFDM/TBST;

Exposure time: 1 minute 30 seconds.

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

Background References

1. Flanagan J.F et al. Double chromodomains cooperate to recognize the methylated histone H3 tail. Nature 438:1181-1185 (2005).
2. Iberg A.N et al. Arginine methylation of the histone H3 tail impedes effector binding. J Biol Chem 283:3006-3010 (2008).