Anti-Histone H3 (mono methyl K4) Antibody

R1110-1

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 within human Histone H3 aa 1-49/136.

Positive control: NCCIT, PC-12, MCF-7, human liver carcinoma tissue, human colon carcinoma tissue, human pancreas tissue, SHSY5Y.

Subcellular location: Nucleus

Database links: SwissProt: P68431 Human | P68433 Mouse | Q497B8 Rat

Recommended Dilutions:

WB	1:500-1:2000
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℃ or -80℃. Avoid repeated freeze / thaw cycles.

Purity: Immunogen affinity purified.

Images

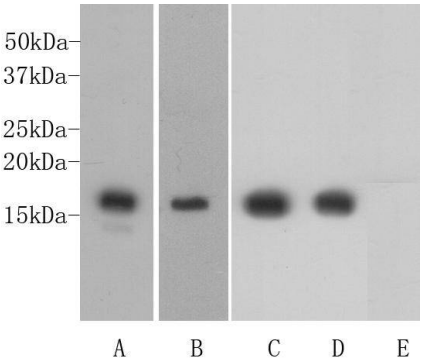

Fig1: Western blot analysis of Histone H3 (mono methyl K4) on different lysates. Proteins were transferred to a PVDF membrane and blocked with 5% BSA in PBS for 1 hour at room temperature. The primary antibody (R1110-1, 1/500) was used in 5% BSA at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:5,000 dilution was used for 1 hour at room temperature.
Positive control:
A: NCCIT
B: purified 293T Histone
C: F9
D: F9 with Histone H3 peptide-unmodifed
E: F9 with Histone H3 peptide-mono methyl K4 lysates using anti-Histone H3(mono methyl K4) polyclonal antibody.

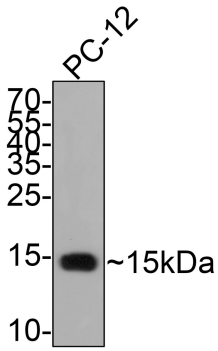

Fig2: Western blot analysis of Histone H3 (mono methyl K4) on PC-12 cell lysates with Rabbit anti-Histone H3 (mono methyl K4) antibody (R1110-1) at 1/500 dilution.

Lysates/proteins at 10 µg/Lane.

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

Exposure time: 2 minutes;

10% 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 (R1110-1) at 1/500 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:300,000 dilution was used for 1 hour at room temperature.

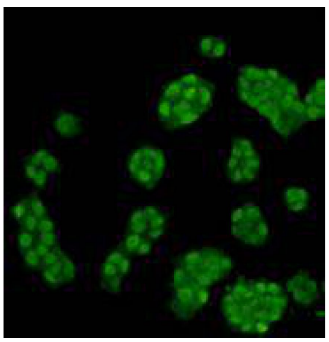

Fig3: ICC staining of Histone H3 (mono methyl K4) in NCCIT cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (R1110-1, 1/100) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

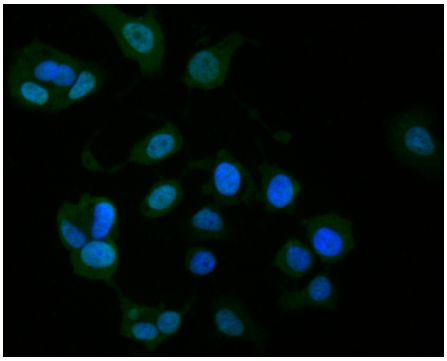

Fig4: Immunocytochemistry analysis of MCF-7 cells labeling Histone H3 (mono methyl K4) with Rabbit anti-Histone H3 (mono methyl K4) antibody (R1110-1) at 1/200 dilution.

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 (mono methyl K4) antibody (R1110-1) at 1/200 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.

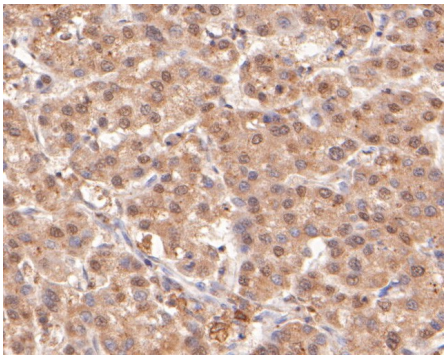

Fig5: Immunohistochemical analysis of paraffin-embedded human liver carcinoma tissue using anti-Histone H3 (mono methyl K4) antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (R1110-1, 1/200) 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.

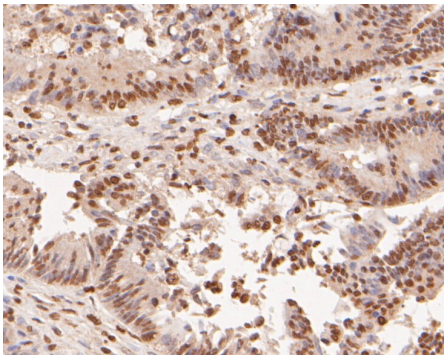

Fig6: Immunohistochemical analysis of paraffin-embedded human colon carcinoma tissue using anti-Histone H3 (mono methyl K4) antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (R1110-1, 1/200) 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.

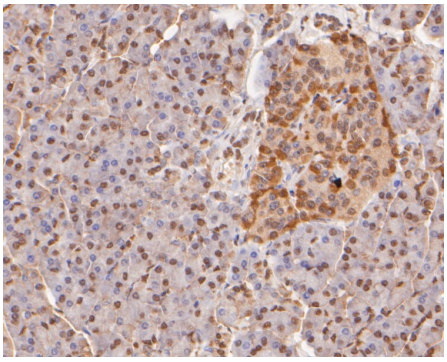

Fig7: Immunohistochemical analysis of paraffin-embedded human pancreas tissue using anti-Histone H3 (mono methyl K4) antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (R1110-1, 1/200) 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.

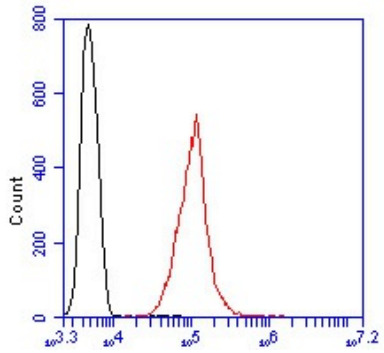

Fig8: Flow cytometric analysis of Histone H3 (mono methyl K4) was done on SHSY5Y cells. The cells were fixed, permeabilized and stained with the primary antibody (R1110-1, 1/50) (red). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor 488-conjugated Goat anti-Rabbit IgG Secondary antibody at 1/1000 dilution for 30 minutes.Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

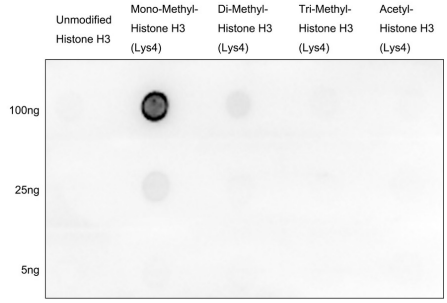

Fig9: Dot blot analysis of Histone H3 (mono methyl K4) on different proteins with Rabbit anti-Histone H3 (mono methyl K4) antibody (R1110-1) at 1/1,000 dilution. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution for 1 hour at room temperature.

Lane 1: Unmodified Histone H3 (negative)
Lane 2: Mono-Methyl-Histone H3 (Lys4) (positive)
Lane 3: Di-Methyl-Histone H3 (Lys4) (negative)
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: 50 seconds.

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)
2. "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)