Anti-Histone H3 Antibody [3-C4]

EM30605

Product Type:	Mouse monoclonal IgG1, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, mIHC, IF-Tissue
Molecular Wt:	Predicted band size: 15 kDa
Clone number:	3-C4

Description: Core component of nucleosome. 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. Nucleosomes wrap and compact DNA into chromatin, limiting DNA accessibility to the cellular machineries which require DNA as a template. 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. Histone H3 is one of the five main histones involved in the structure of chromatin in eukaryotic cells. Featuring a main globular domain and a long N-terminal tail, H3 is involved with the structure of the nucleosomes of the 'beads on a string' structure. Histone proteins are highly post-translationally modified however Histone H3 is the most extensively modified of the five histones. The term "Histone H3" alone is purposely ambiguous in that it does not distinguish between sequence variants or modification state. Histone H3 is an important protein in the emerging field of epigenetics, where its sequence variants and variable modification states are thought to play a role in the dynamic and long term regulation of genes.

Immunogen: Synthetic peptide within C-terminal residues of human Histone H3.

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, mouse kidney, human liver tissue, human testis tissue, mouse testis tissue, rat testis tissue, rat brain tissue.

Subcellular location: Nucleus.

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

Recommended Dilutions:

WB	1:10,000
IHC-P	1:1,000-1:2,000
mIHC	1:500
IF-Tissue	1:200-1:400

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: Protein A affinity purified.

Images

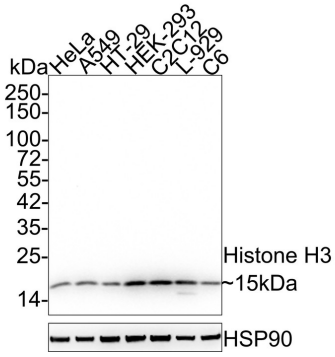

Fig1: Western blot analysis of Histone H3 on different lysates with Mouse anti-Histone H3 antibody (EM30605) at 1/10,000 dilution.

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: 30 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 (EM30605) at 1/10,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1/50,000 dilution was used for 1 hour at room temperature.

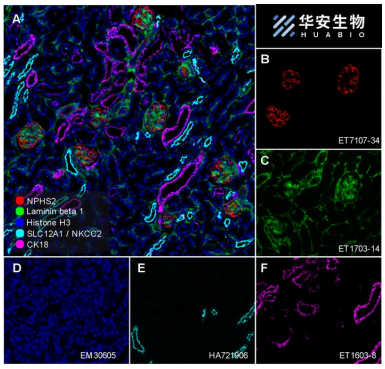

Fig2: Fluorescence multiplex immunohistochemical analysis of mouse kidney (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-NPHS2 (ET7107-34, Red), anti-Laminin beta 1 (ET1703-14, Green), anti-Histone H3 (EM30605, Blue), anti-SLC12A1 / NKCC2 (HA721906, Cyan) and anti-CK18 (ET1603-8, Magenta) on kidney. HRP Conjugated UltraPolymer Goat Polyclonal Antibody HA1119/HA1120 was used as a secondary antibody. The immunostaining was performed with the Sequential Immuno-staining Kit (IRISKit™MH010101, www.luminiris.cn). The section was incubated in five rounds of staining: in the order of ET7107-34 (1/1,000 dilution), ET1703-14 (1/1,000 dilution), EM30605 (1/500 dilution), HA721906 (1/3,000 dilution) and ET1603-8 (1/3,000 dilution) for 20 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 30 mins at 95℃. Image acquisition was performed with Olympus VS200 Slide Scanner.

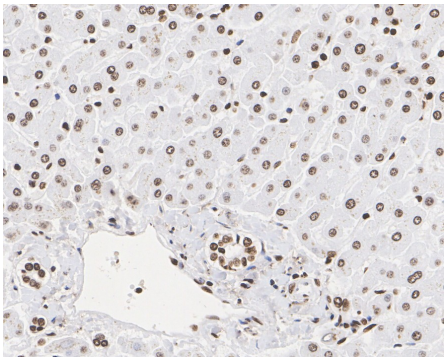

Fig3: Immunohistochemical analysis of paraffin-embedded human liver tissue with Mouse anti-Histone H3 antibody (EM30605) at 1/2,000 dilution.

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 (EM30605) at 1/2,000 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.

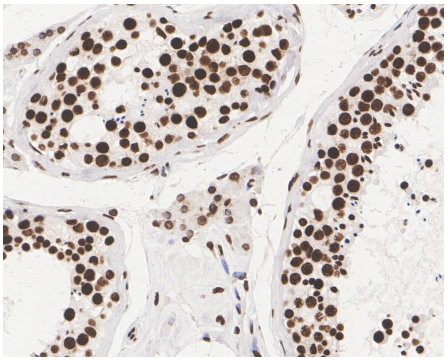

Fig4: Immunohistochemical analysis of paraffin-embedded human testis tissue with Mouse anti-Histone H3 antibody (EM30605) at 1/1,000 dilution.

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 (EM30605) 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.

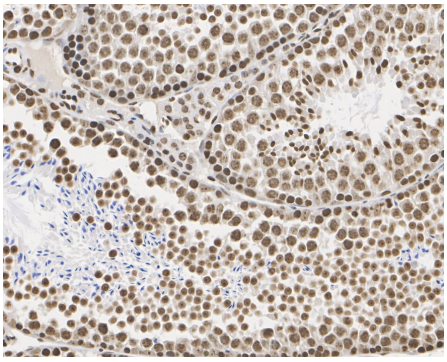

Fig5: Immunohistochemical analysis of paraffin-embedded mouse testis tissue with Mouse anti-Histone H3 antibody (EM30605) at 1/2,000 dilution.

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 (EM30605) at 1/2,000 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.

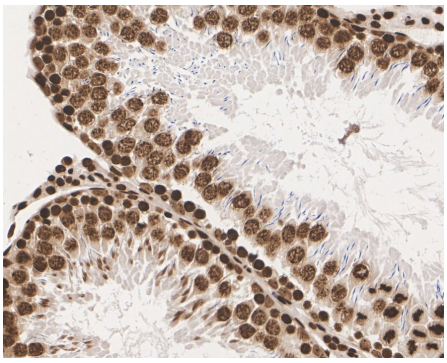

Fig6: Immunohistochemical analysis of paraffin-embedded rat testis tissue with Mouse anti-Histone H3 antibody (EM30605) at 1/1,000 dilution.

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 (EM30605) 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.

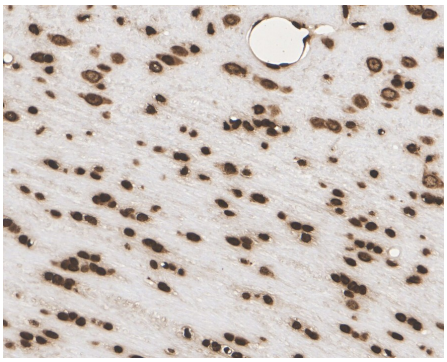

Fig7: Immunohistochemical analysis of paraffin-embedded rat brain tissue with Mouse anti-Histone H3 antibody (EM30605) at 1/2,000 dilution.

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 (EM30605) at 1/2,000 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.

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)