Anti-Histone H3 (tri methyl K4) Antibody [PSH25-68]

HA724555

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat, Green monkey
Applications:	WB, IF-Cell, IHC-P, FC(Intra), ChIP
Molecular Wt:	Predicted band size: 15 kDa
Clone number:	PSH25-68

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).

Positive control: HeLa (Human cervical adenocarcinoma cell) cell lysate, U-2 OS (Human osteosarcoma cell) cell lysate, NIH/3T3 (Mouse fibroblast) cell lysate, C2C12 (Mouse myoblast) cell lysate, C6 (Rat glioma cell) cell lysate, PC-12 (Rat pheochromocytoma cell (undifferentiated)) cell lysate, COS-1 (African green monkey kidney fibroblast) cell lysate.

Subcellular location: Nucleus.

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

Recommended Dilutions:

WB	1:2,000
IF-Cell	1:500
IHC-P	1:50-1:100
FC(Intra)	1:1,000
ChIP	Use 0.5~2 μg for 25 μg of chromatin.

Storage Buffer: 1*PBS (pH7.4), 0.1% BSA, 40% Glycerol, 0.2% Proclean 950.

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

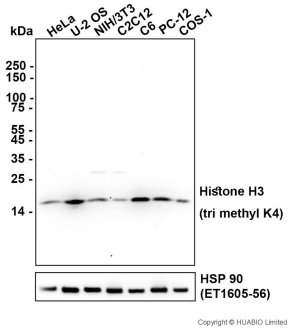

Fig1: Western blot analysis of Histone H3 (tri methyl K4) on different lysates with Rabbit anti-Histone H3 (tri methyl K4) antibody (HA724555) at 1/2,000 dilution.

Lane 1: HeLa (Human cervical adenocarcinoma cell) cell lysate
Lane 2: U-2 OS (Human osteosarcoma cell) cell lysate
Lane 3: NIH/3T3 (Mouse fibroblast) cell lysate
Lane 4: C2C12 (Mouse myoblast) cell lysate
Lane 5: C6 (Rat glioma cell) cell lysate
Lane 6: PC-12 (Rat pheochromocytoma cell (undifferentiated)) cell lysate
Lane 7: COS-1 (African green monkey kidney fibroblast) cell lysate

Lysates/proteins at 20 µg/Lane.
Exposure time: 1 minute 30 seconds; ECL: K1801

Blocking: 5% NFDM/TBST, 1 hour at room temperature
Primary antibody: HA724555, 1/2,000 in primary antibody dilution buffer (K1803), overnight at 4 °C
Secondary antibody: Goat anti-Rabbit IgG-HRP (HA1001), 1/50,000 in 5% NFDM/TBST, 1 hour at room temperature

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

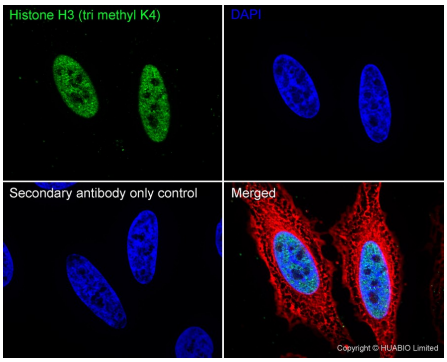

Fig2: Application: Immunocytochemistry (IF-cell)

Species: Human
Sample: HeLa (Human cervical adenocarcinoma cell)

Fixation: 4% Paraformaldehyde, 15 minutes at room temperature.
Permeabilization: 0.1% Triton X-100, 15 minutes at room temperature.
Blocking: 1% BSA + 10% normal goat serum, 1 hour at room temperature.
Antibody dilution buffer: 1% BSA in PBST.

Primary antibody: HA724555, 1/500, overnight at 4°C.
Secondary antibody: Goat Anti-Rabbit IgG (iFluor™ 488, HA1121), 45 minutes at room temperature.
Counterstain: Beta tubulin (HA601187, Red), 1/100, overnight at 4℃. The nuclear counterstain was DAPI (Blue).

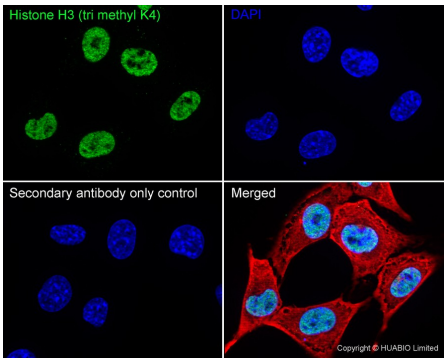

Fig3: Application: Immunocytochemistry (IF-cell)

Species: Mouse
Sample: NIH/3T3 (Mouse fibroblast)

Fixation: 4% Paraformaldehyde, 15 minutes at room temperature.
Permeabilization: 0.1% Triton X-100, 15 minutes at room temperature.
Blocking: 1% BSA + 10% normal goat serum, 1 hour at room temperature.
Antibody dilution buffer: 1% BSA in PBST.

Primary antibody: HA724555, 1/500, overnight at 4°C.
Secondary antibody: Goat Anti-Rabbit IgG (iFluor™ 488, HA1121), 45 minutes at room temperature.
Counterstain: Beta tubulin (HA601187, Red), 1/100, overnight at 4℃. The nuclear counterstain was DAPI (Blue).

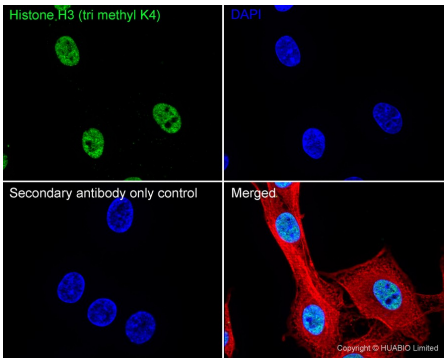

Fig4: Application: Immunocytochemistry (IF-cell)

Species: Rat
Sample: C6 (Rat glioma cell)

Fixation: 4% Paraformaldehyde, 15 minutes at room temperature.
Permeabilization: 0.1% Triton X-100, 15 minutes at room temperature.
Blocking: 1% BSA + 10% normal goat serum, 1 hour at room temperature.
Antibody dilution buffer: 1% BSA in PBST.

Primary antibody: HA724555, 1/500, overnight at 4°C.
Secondary antibody: Goat Anti-Rabbit IgG (iFluor™ 488, HA1121), 45 minutes at room temperature.
Counterstain: Beta tubulin (HA601187, Red), 1/100, overnight at 4℃. The nuclear counterstain was DAPI (Blue).

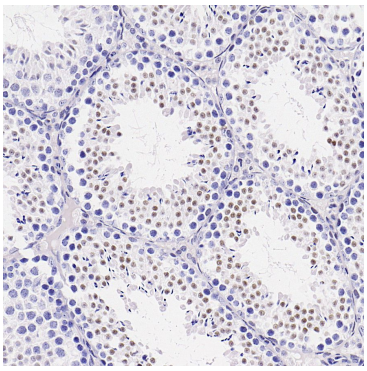

Fig5: Application: Immunohistochemistry (IHC-P)

Species: Mouse
Tissue: Testis
Sample: Paraffin-embedded section

Antigen retrieval: Heat-mediated, Tris-EDTA buffer (pH 9.0), 20 minutes at 95°C.

Wash buffer: 1× TBST
Endogenous peroxidase blocking: 3% H_2O_2, 10 minutes at room temperature.
Blocking: 1% BSA + 10% normal goat serum, 10 minutes at room temperature.
Primary antibody: HA724555, 1/100, 1 hour at room temperature.
Secondary antibody: HA1119, 20 minutes at room temperature.

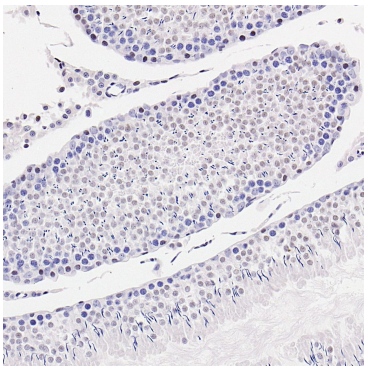

Fig6: Application: Immunohistochemistry (IHC-P)

Species: Rat
Tissue: Testis
Sample: Paraffin-embedded section

Antigen retrieval: Heat-mediated, Tris-EDTA buffer (pH 9.0), 20 minutes at 95°C.

Wash buffer: 1× TBST
Endogenous peroxidase blocking: 3% H_2O_2, 10 minutes at room temperature.
Blocking: 1% BSA + 10% normal goat serum, 10 minutes at room temperature.
Primary antibody: HA724555, 1/100, 1 hour at room temperature.
Secondary antibody: HA1119, 20 minutes at room temperature.

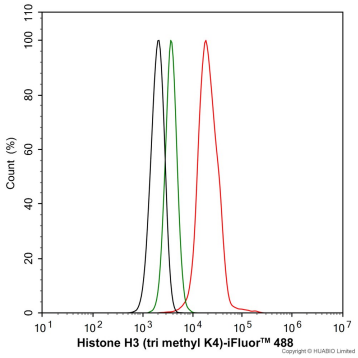

Fig7: Application: Flow Cytometry (Intra)

Species: Human
Sample: HeLa (Human cervix adenocarcinoma epithelial cell)

Fixation: 4% Paraformaldehyde, 15 minutes at room temperature.
Permeabilization: 0.1% Tween-20, 15 minutes at room temperature.
Blocking: 1% BSA + 10% normal goat serum, 15 minutes at room temperature.
Antibody dilution buffer: 1x PBS.

Primary antibody: HA724555 (1/1,000, Red) compared with Rabbit IgG Isotype Control (HA722127, Green), 15 minutes at room temperature.
Secondary antibody: Goat Anti-Rabbit IgG (iFluor™ 488, HA1121), 15 minutes at room temperature.

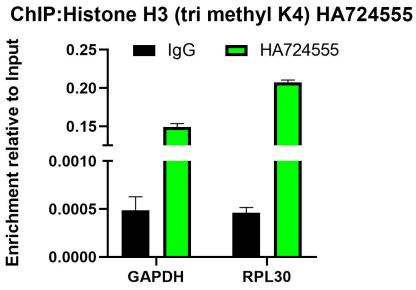

Fig8: Chromatin immunoprecipitations were performed with cross-linked chromatin from HeLa cells with Histone H3 (tri methyl K4) (HA724555) / Competitor's antibody / Normal Rabbit IgG according to the ChIP protocol. The enriched DNA was quantified by real-time PCR using indicated primers. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin, which is equivalent to one.

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

Background References

1. Caeiro LD et al. Histone H3 mutations and their impact on genome stability maintenance. Biochem Soc Trans. 2024 Oct
2. Young D et al. The role of histone H3 lysine demethylases in glioblastoma. Cancer Metastasis Rev. 2023 Jun