# Anti-Histone H4 Antibody [SD209-04] ET1612-43

#### **Product Type:** Recombinant Rabbit monoclonal IgG, primary antibodies Species reactivity: Human, Mouse, Rat WB, IF-Cell, IF-Tissue, IHC-P, ChIP **Applications:** Molecular Wt: Predicted band size: 11 kDa SD209-04 **Clone number:** 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 fiber. Two molecules of each of the four core histones (H2A, H2B, H3, and H4) form the octamer; formed of two H2A-H2B dimers and two H3-H4 dimers, forming two nearly symmetrical halves by tertiary structure. Over 80% of nucleosomes contain the linker Histone H1, derived from an intronless gene, that interacts with linker DNA between nucleosomes and mediates compaction into higher order chromatin. Histones are subject to posttranslational modification by enzymes primarily on their Nterminal tails, but also in their globular domains. Such modifications include methylation, citrullination, acetylation, phosphorylation, sumoylation, ubiquitination and ADP-ribosylation. Immunogen: Synthetic peptide within C-terminal Human Histone H4 aa 54-103 / 103. **Positive control:** HeLa cell lysate, HepG2 cell lysate, Jurkat cell lysate, C2C12 cell lysate, NIH/3T3 cell lysate, C6 cell lysate, PC-12 cell lysate, human kidney tissue, human testis tissue, mouse kidney tissue, mouse testis tissue, rat kidney tissue, rat testis tissue, PANC-1. Subcellular location: Nucleus, Chromosome. Database links: SwissProt: P62805 Human | P62806 Mouse | P62804 Rat **Recommended Dilutions:** WB 1:2.000 IF-Cell 1:50-1:200 IF-Tissue 1:500-1:2,000 IHC-P 1:1:20.000 ChIP Use 0.5~2 µg for 25 µg of chromatin. **Storage Buffer:** 1\*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide. Storage Instruction: Store at +4 $^{\circ}$ C after thawing. Aliquot store at -20 $^{\circ}$ C or -80 $^{\circ}$ C. Avoid repeated freeze / thaw cycles. **Purity:** Protein A affinity purified.

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Orders:0086-571-88062880

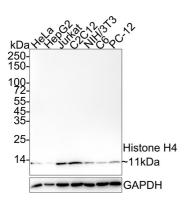
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#### Images



**Fig1:** Western blot analysis of Histone H4 on different lysates with Rabbit anti-Histone H4 antibody (ET1612-43) at 1/2,000 dilution.

Lane 1: HeLa cell lysate Lane 2: HepG2 cell lysate Lane 3: Jurkat cell lysate Lane 4: C2C12 cell lysate Lane 5: NIH/3T3 cell lysate Lane 6: C6 cell lysate Lane 7: PC-12 cell lysate

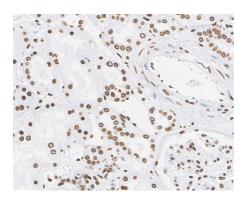
Lysates/proteins at 20 µg/Lane.

Predicted band size: 11 kDa Observed band size: 11 kDa

Exposure time: 58 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 (ET1612-43) at 1/2,000 dilution was used in 5% NFDM/TBST at  $4^{\circ}$ C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.



**Fig2:** Immunohistochemical analysis of paraffin-embedded human kidney tissue with Rabbit anti-Histone H4 antibody (ET1612-43) at 1/20,000 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<sub>2</sub>O and PBS, and then probed with the primary antibody (ET1612-43) at 1/20,000 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.

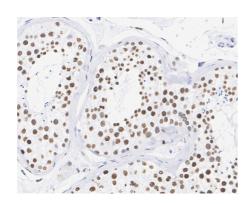
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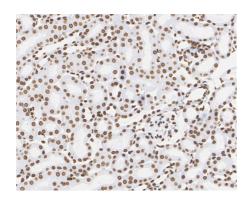
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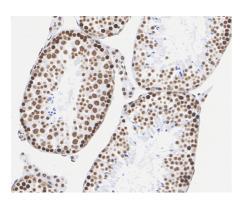
**Fig3:** Immunohistochemical analysis of paraffin-embedded human testis tissue with Rabbit anti-Histone H4 antibody (ET1612-43) at 1/20,000 dilution.

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<sub>2</sub>O and PBS, and then probed with the primary antibody (ET1612-43) at 1/20,000 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 mouse kidney tissue with Rabbit anti-Histone H4 antibody (ET1612-43) at 1/20,000 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<sub>2</sub>O and PBS, and then probed with the primary antibody (ET1612-43) at 1/20,000 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 Rabbit anti-Histone H4 antibody (ET1612-43) at 1/20,000 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<sub>2</sub>O and PBS, and then probed with the primary antibody (ET1612-43) at 1/20,000 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.

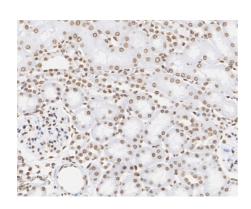
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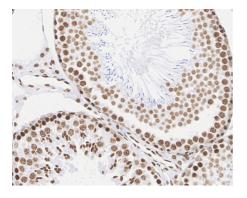
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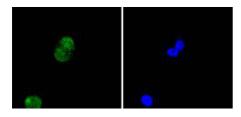
**Fig6:** Immunohistochemical analysis of paraffin-embedded rat kidney tissue with Rabbit anti-Histone H4 antibody (ET1612-43) at 1/20,000 dilution.

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<sub>2</sub>O and PBS, and then probed with the primary antibody (ET1612-43) at 1/20,000 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.

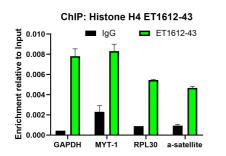


**Fig7:** Immunohistochemical analysis of paraffin-embedded rat testis tissue with Rabbit anti-Histone H4 antibody (ET1612-43) at 1/20,000 dilution.

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<sub>2</sub>O and PBS, and then probed with the primary antibody (ET1612-43) at 1/20,000 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:** ICC staining of Histone H4 in PANC-1 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 (ET1612-43, 1/50) 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).



**Fig9:** Chromatin immunoprecipitations were performed with crosslinked chromatin from HeLa cells with Histone H4 (ET1612-43) or 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.

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

#### **Background References**

- 1. Westhorpe FG et al. A cell-free CENP-A assembly system defines the chromatin requirements for centromere maintenance. J Cell Biol 209:789-801 (2015).
- 2. Ghule PN et al. Fidelity of histone gene regulation is obligatory for genome replication and stability. Mol Cell Biol 34:2650-9 (2014).

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Applications:WB=Western blot IHC-P=Immunohistochemistry (paraffin) IF-Cell=Immunofluorescence (Cell) IF-Tissue=Immunofluorescence (Tissue) FC=Flow cytometry IP=Immunoprecipitation

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