Anti-Phospho-Histone H3 (S10) Antibody [SA31-01] - BSA and Azide free

HA750020

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IHC-P, IP, FC, ChIP
Molecular Wt:	Predicted band size: 15 kDa
Clone number:	SA31-01

Description: 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 phospho-peptide corresponding to residues surrounding Ser10 of human Histone H3.

Positive control: HeLa treated with 50nM Calyculin A for 30 minutes whole cell lysate, NIH/3T3 treated with 100nM Calyculin A for 30 minutes whole cell lysate, C6 treated with 100nM Calyculin A for 30 minutes whole cell lysate, HeLa, human kidney tissue, mouse kidney tissue, rat kidney tissue.

Subcellular location: Nucleus, Chromosome

Database links: SwissProt: P68431 Human | P84243 Human | Q16695 Human | Q6NXT2 Human | Q71DI3 Human | P84244 Mouse | P84245 Rat

Recommended Dilutions:

WB	1:2,000-1:10,000
IF-Cell	1:5,000
IHC-P	1:100
IP	Use at an assay dependent concentration.
FC	1:1,000
ChIP	Use 0.5~2 μg for 25 μg of chromatin.

Storage Buffer: PBS (pH7.4).

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

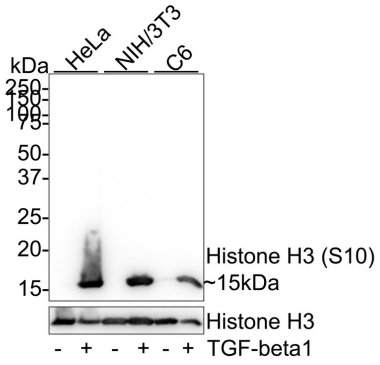

Fig1: Western blot analysis of Phospho-Histone H3 (S10) on different lysates with Rabbit anti-Phospho-Histone H3 (S10) antibody (HA750020) at 1/5,000 dilution.

Lane 1: HeLa whole cell lysate (20 µg/Lane)
Lane 2: HeLa treated with 50nM Calyculin A for 30 minutes whole cell lysate (20 µg/Lane)
Lane 3: NIH/3T3 whole cell lysate (20 µg/Lane)
Lane 4: NIH/3T3 treated with 100nM Calyculin A for 30 minutes whole cell lysate (20 µg/Lane)
Lane 5: C6 whole cell lysate (20 µg/Lane)
Lane 6: C6 treated with 100nM Calyculin A for 30 minutes whole cell lysate (20 µg/Lane)

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

Exposure time: 10 seconds; ECL: K1801;
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 (HA750020) at 1/5,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:50,000 dilution was used for 1 hour at room temperature.

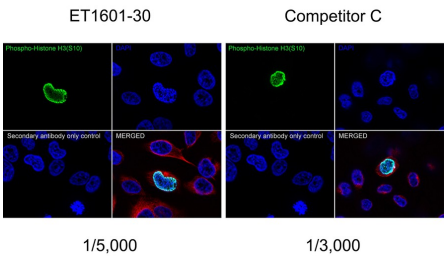

Fig2: Immunocytochemistry analysis of HeLa cells labeling Phospho-Histone H3 (S10) with Rabbit anti-Phospho-Histone H3 (S10) antibody (HA750020) at 1/5,000 dilution and competitor's antibody at 1/3,000 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-Phospho-Histone H3 (S10) antibody (HA750020) at 1/5,000 dilution and competitor's antibody at 1/3,000 dilution in 1% BSA in PBST 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.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

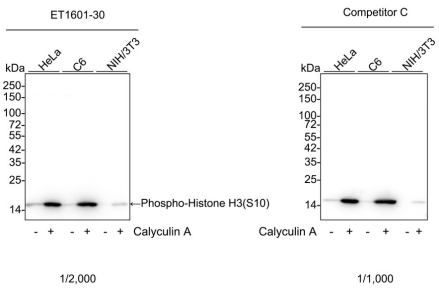

Fig3: Western blot analysis of Phospho-Histone H3 (S10) on different lysates with Rabbit anti-Phospho-Histone H3 (S10) antibody (HA750020) at 1/2,000 dilution and competitor's antibody at 1/1,000 dilution.

Lane 1: HeLa whole cell lysate
Lane 2: HeLa treated with 50nM Calyculin A for 30 minutes whole cell lysate
Lane 3: C6 whole cell lysate
Lane 4: C6 treated with 100ng/mL Calyculin A for 1 hour whole cell lysate
Lane 5: NIH/3T3 whole cell lysate
Lane 6: NIH/3T3 starved for 4 hours, then treated with 100nM Calyculin A for 30 minutes whole cell lysate

Lysates/proteins at 15 µg/Lane.

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

Exposure time: 15 seconds; ECL: K1801;

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 (HA750020) at 1/2,000 dilution and competitor's antibody at 1/1,000 dilution were used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

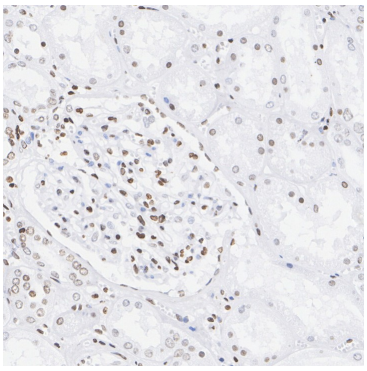

Fig4: Immunohistochemical analysis of paraffin-embedded human kidney tissue with Rabbit anti-Phospho-Histone H3 (S10) antibody (HA750020) at 1/100 dilution.

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 (HA750020) at 1/100 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.

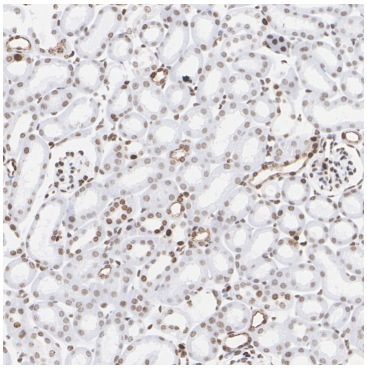

Fig5: Immunohistochemical analysis of paraffin-embedded mouse kidney tissue with Rabbit anti-Phospho-Histone H3 (S10) antibody (HA750020) at 1/100 dilution.

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 (HA750020) at 1/100 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.

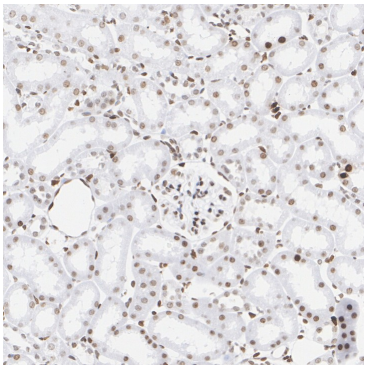

Fig6: Immunohistochemical analysis of paraffin-embedded rat kidney tissue with Rabbit anti-Phospho-Histone H3 (S10) antibody (HA750020) at 1/100 dilution.

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 (HA750020) at 1/100 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.

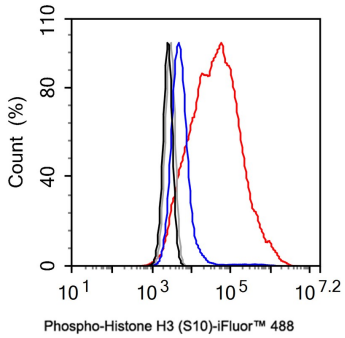

Fig7: Flow cytometric analysis of HeLa untreated cells and HeLa treated with 100nM Calyculin A for 30 minutes cells labeling Phospho-Histone H3 (S10).

Cells were fixed and permeabilized. Then stained with the primary antibody (HA750020, 1μg/mL) (HeLa untreated，blue; HeLa treated, red) compared with Rabbit IgG Isotype Control (grey). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

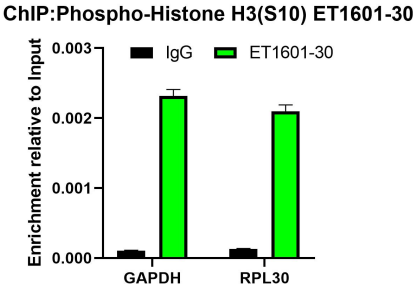

Fig8: Chromatin immunoprecipitations were performed with cross-linked chromatin from HeLa cells treated with 100ng/mL Nocodazole for 18 hours with Phospho-Histone H3 (S10) (HA750020) or Normal Rabbit IgG according to the ChIP protocol. The enriched DNA was quantified by real-time PCR using indicated 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.

Background References

1. Hammond SL et al. Mitotic phosphorylation of histone H3 threonine 80. Cell Cycle 13:440-52 (2014).
2. Martin HL et al. High-content, high-throughput screening for the identification of cytotoxic compounds based on cell morphology and cell proliferation markers. PLoS One 9:e88338 (2014).