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Product datasheet for TA347173

H3FA (HIST1H3A) Rabbit Polyclonal Antibody

Product data:

Product Type:	Primary Antibodies
Applications:	Dot, ELISA, IF, IP, WB
Recommended Dilution:	ChIP (1 µl/ChIP); ELISA (1:1,000 ?? 1:5,000) ; Dot blotting (1:20,000); Western blotting (1:500) ; Immunofluorescence (1:200); Immunoprecipitation (5 µl/IP)
Reactivity:	Human
Host:	Rabbit
Isotype:	IgG
Clonality:	Polyclonal
Immunogen:	The immunogen for anti-H3S10p antibody: histone H3 containing the phosphorylated serine 10 (H3S10p), using a KLH-conjugated synthetic peptide.
Concentration:	lot specific
Purification:	Whole antiserum from rabbit containing 0.05% azide.
Conjugation:	Unconjugated
Storage:	Store at -20°C as received.
Stability:	Stable for 12 months from date of receipt.
Gene Name:	histone cluster 1, H3a
Database Link:	NP_003520 Entrez Gene 8350 Human P68431

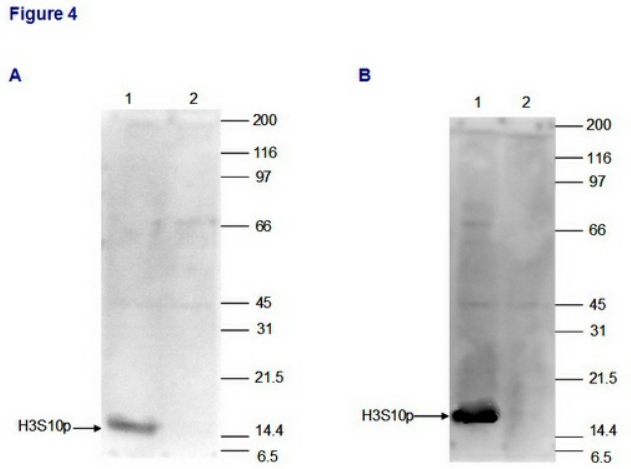
View online »

Background: Histones are the main constituents of the protein part of chromosomes of eukaryotic cells. They are rich in the amino acids arginine and lysine and have been greatly conserved during evolution. Histones pack the DNA into tight masses of chromatin. Two core histones of each class H2A, H2B, H3 and H4 assemble and are wrapped by 146 base pairs of DNA to form one octameric nucleosome. Histone tails undergo numerous post-translational modifications, which either directly or indirectly alter chromatin structure to facilitate transcriptional activation or repression or other nuclear processes. In addition to the genetic code, combinations of the different histone modifications reveal the so-called "histone code". Histone methylation and demethylation is dynamically regulated by respectively histone methyl transferases and histone demethylases. Phosphorylation of H3S10 is associated with mitosis.

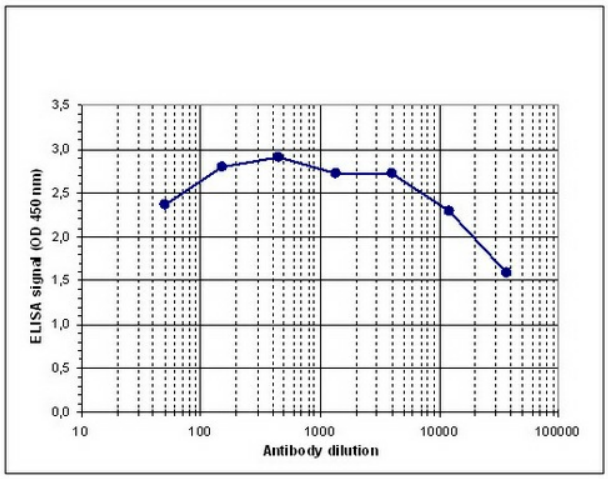
Synonyms: A; H3; H3FA

Protein Pathways: Systemic lupus erythematosus

Product images:

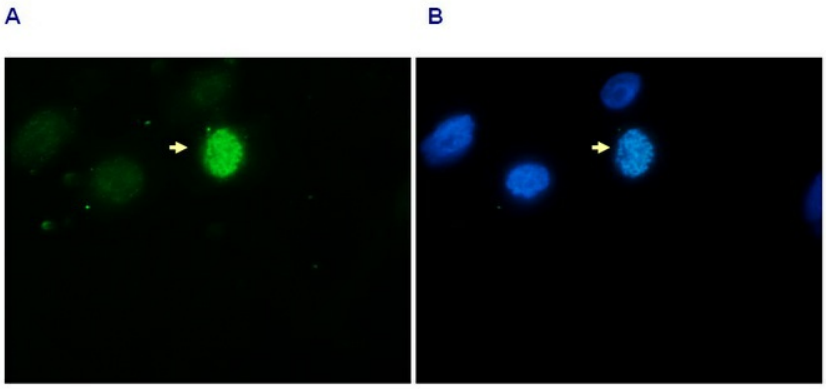


WB using the antibody against H3S10p diluted 1:500 in TBS-Tween containing 5% skimmed milk. The position of the protein of interest is indicated on the left; the marker (in kDa) is shown on the right. The result of the Western analysis with the antibody is shown in lane 1; lane 2 shows the same analysis after incubation of the antibody with 750 pmol blocking peptide for 1 hour at room temperature.

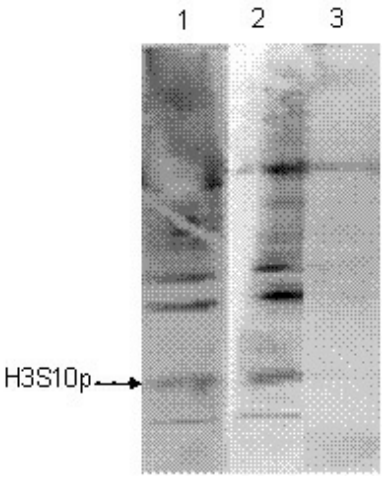


Determination of the titer To determine the titer, an ELISA was performed using a serial dilution of the antibody against human H3S10p. The antigen used was a peptide containing the histone modification of interest. By plotting the absorbance against the antibody dilution (Figure 2), the titer of the antibody was estimated to be 1:35,000.

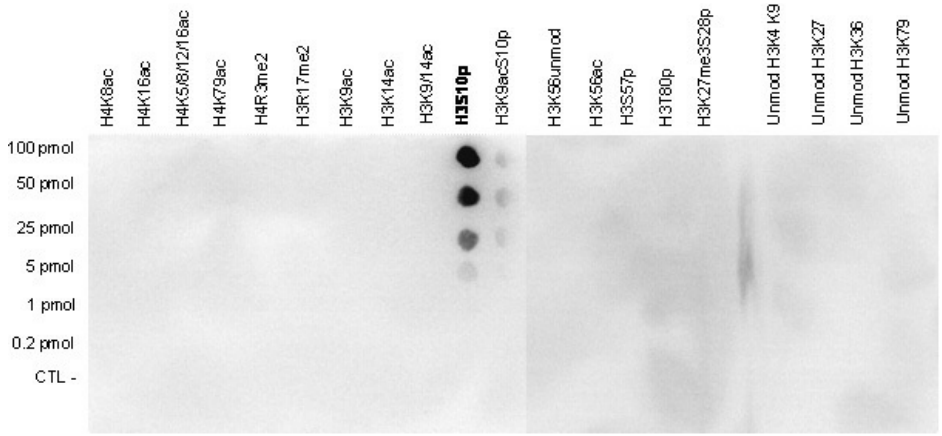
Figure 5



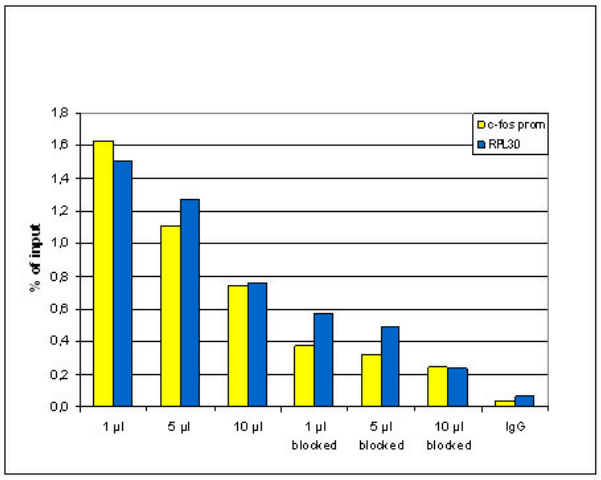
HeLa cells were fixed with formaldehyde, permeabilized with sodium citrate and Triton X100 and blocked with PBS with 2.5% BSA. (A) Cells were labelled with the H3S10p antibody (at 1:200 and incubated for 1 hr at RT) followed by goat anti-rabbit antibody conjugated to DyLight 488. (B) The nuclei were stained with DAPI. Phosphorylation of H3 on serine 10 occurs on condensed chromosomes during mitosis. This explains the dense staining of one of the cells (indicated with an arrow).



HeLa cells were treated with colcemid to block the cell cycle in metaphase and were fixed with formaldehyde. Chromatin from 10,000 cells was sheared and used for IP. IP was performed with 5 ul of the ab. The IP'd proteins were analysed by WB with the antibody diluted 1:500 in TBS-Tween containing 5% milk. Lane 1 shows the result of the IP; a positive control (sheared chromatin from 10,000 cells) and a negative IP control (no antibody added) are shown in lane 2 and 3, respectively.



Dot Blot was performed to test the cross reactivity with peptides containing other modifications of histone H3 and H4 and with peptides containing unmodified sequences from histone H3. One hundred to 0.2 pmol of the peptide containing the respective histone modification were spotted on a membrane. Dilution: 1:20,000. Image shows a high specificity of the ab for the modification of interest. Note that the antibody does not recognize the H3S10p modification if the H3K9ac modification is present.



ChIP assays were performed using HeLa cells treated with colcemid (sheared chromatin from 10,000 cells). Titration of 1, 5, and 10ul antibody per ChIP was performed after incubation with 5 nM blocking peptide for 1 hr at RT. IgG (5 ug/IP) was used as negative control. qPCR primers were for the promoter of the active genes c-fos and RPL30. Image shows the recovery, expressed as a % of input (the relative amount of IP'd DNA compared to input DNA after qPCR analysis).