OriGene Technologies, Inc.
9620 Medical Center Drive, Ste 200
Rockville, MD 20850, US
Phone: +1-888-267-4436
https://www.origene.com
techsupport@origene.com
EU: info-de@origene.com
CN: techsupport@origene.cn

Product datasheet for TA347196

H3FA (HIST1H3A) Rabbit Polyclonal Antibody

Product data:

Product Type:	Primary Antibodies
Applications:	Dot, ELISA, IF, WB
Recommended Dilution:	ChIP (2 µg per IP); ELISA (1:100); Dot blotting (1:20,000); Western blotting (1:1,000); Immunofluorescence (1:2,000)
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:	Affinity purified polyclonal antibody in PBS containing 0.05% azide and 0.05% ProClin 300.
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

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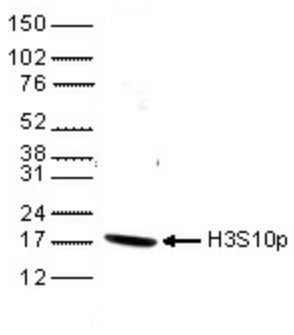
Background: Histones are the main constituents of the protein part of chromosomes of eukaryotic cells. They are rich in the amino acids arginine and lysine and have been greatly conserved during evolution. Histones pack the DNA into tight masses of chromatin. Two core histones of each class H2A, H2B, 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.

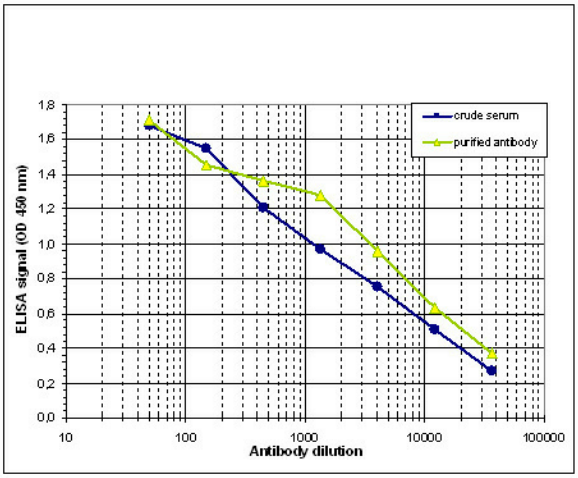
Synonyms: A; H3; H3FA

Protein Pathways: Systemic lupus erythematosus

Product images:



WB using the antibody against H3S10p diluted 1:1,000 in TBS-Tween containing 5% skimmed milk. The position of the protein of interest is indicated on the right; the marker (in kDa) is shown on the left.



Determination of the titer To determine the titer, an ELISA was performed using a serial dilution of the antibody against H3S10p and the crude serum. 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 1:5, 200.

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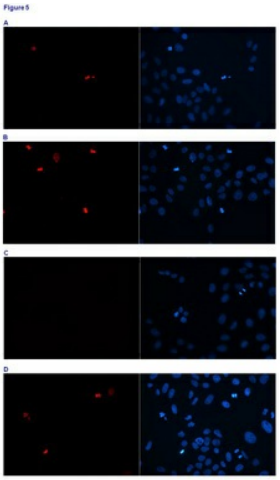
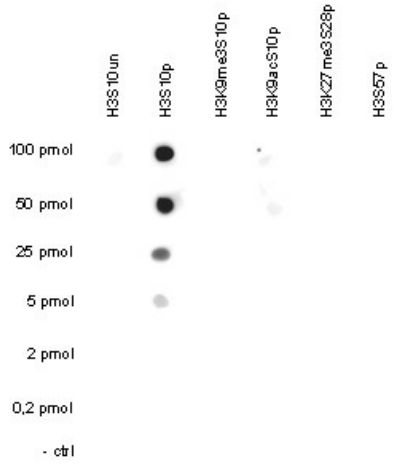
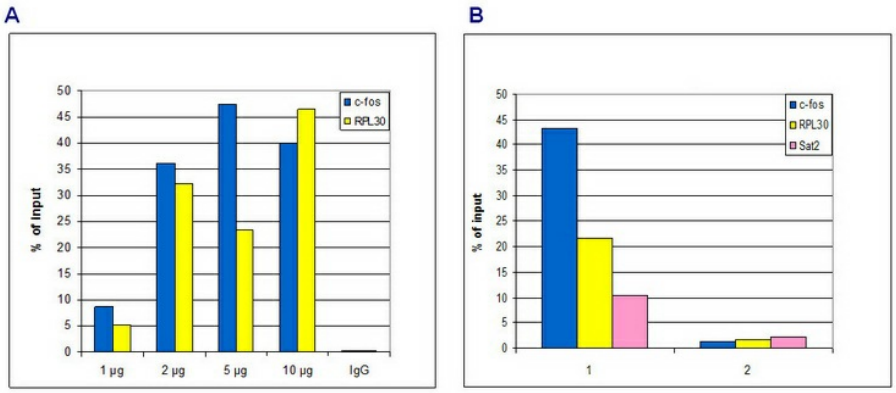


Human U2OS cells were fixed with 3.7% formaldehyde for 20' at RT, followed by a 20' permeabilization with 0.5% Triton X- 100 in PBS and blocked PBS/TX-100 with 5% normal goat serum. A: cells were labeled with the ab (left) at 1:2,000 followed by anti-rabbit conjugated to Alexa568 or with DAPI (right). B, C and D: staining with the antibody after incubation with 2 uM blocking peptide containing the unmodified H3S10 sequence, the phosphorylated H3S10 and the phosphorylated H3T11, respectively.



Dot Blot to test the cross reactivity of the ab against H3S10p with peptides containing other modifications of histone H3 or the unmodified H3S10 sequence. One hundred to 0.2 pmol of the peptide containing the respective histone modification were spotted on a membrane. The antibody was at 1:20,000. Image shows a high specificity of the antibody for the modification of interest. Note that the antibody does not recognize the H3S10p modification if the neighboring K9 is acetylated or trimethylated.

Figure 1



ChIP assays using HeLa cells treated with colcemid to block the cells in metaphase (sheared chromatin from 10,000 cells). A titration of the antibody consisting of 1, 2, 5, and 10 ug per ChIP was performed using 2 ug of H3S10p antibody and sheared chromatin from 10,000 HeLa cells treated with colcemid (sample 1) or from 10,000 untreated cells (sample 2). qPCR primers were for the promoter of the active genes c-fos and RPL30, and for the Sat2 satellite repeat region.

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