

Product datasheet for **TA347175**

H3FA (HIST1H3A) Rabbit Polyclonal Antibody

Product data:

Product Type:	Primary Antibodies
Applications:	Dot, ELISA, IF, WB
Recommended Dilution:	ChIP (5 µl/ChIP); ELISA (1:1,000 ?? 1:10,000); Dot blotting (1:1,000); Western blotting (1:500); Immunofluorescence (1:500)
Reactivity:	Human
Host:	Rabbit
Isotype:	IgG
Clonality:	Polyclonal
Immunogen:	The immunogen for anti-H3K9me3S10p antibody: histone H3 containing trimethylated lysine 9 and the phosphorylated serine 10 (H3K9me3S10p), 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



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Background:

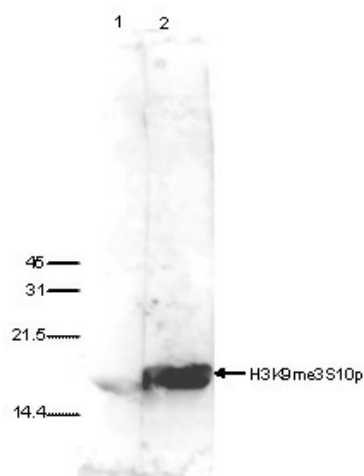
Histones are the main constituents of the protein part of chromosomes of eukaryotic cells. They are rich in the amino acids arginine and lysine and have been greatly conserved during evolution. Histones pack the DNA into tight masses of chromatin. Two core histones of each class H2A, H2B, H3 and H4 assemble 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.

Synonyms:

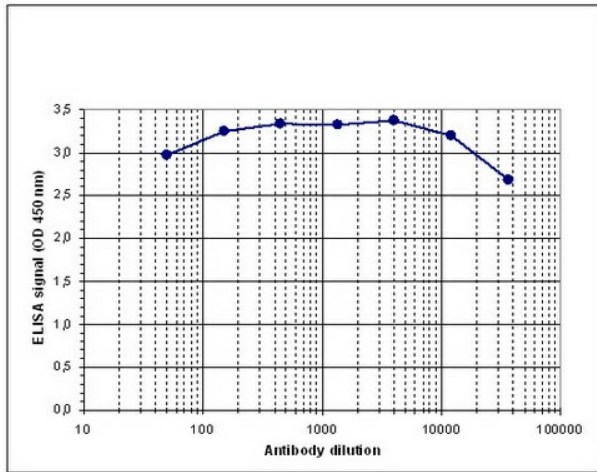
A; H3; H3FA

Protein Pathways:

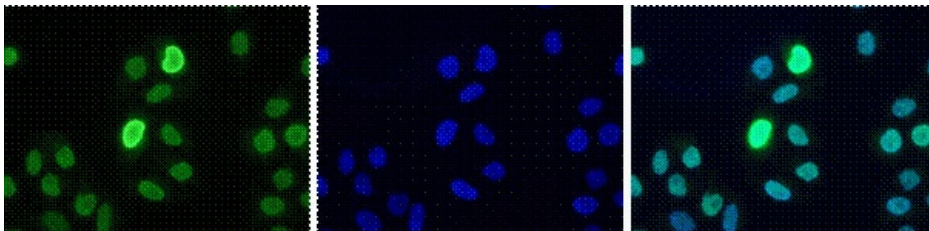
Systemic lupus erythematosus

Product images:

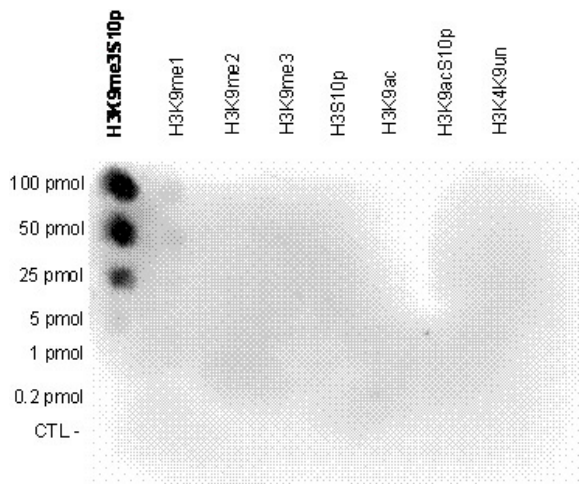
WB with the antibody against H3K9me3S10p diluted 1:500 in TBS-Tween containing 5% skimmed milk. The position of the protein of interest is indicated on the right; the marker (in kDa) is shown on the left. The result of the Western analysis with the antibody is shown in lane 2; lane 1 shows the same analysis after incubation of the antibody with 5 nmol blocking peptide for 1 hour at room temperature.



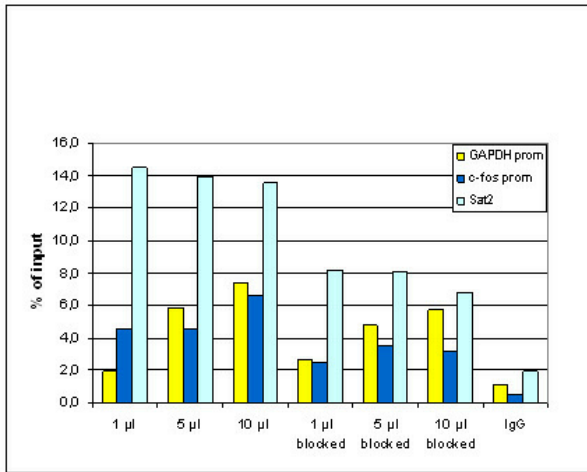
Determination of the titer To determine the titer, an ELISA was performed using a serial dilution of the antibody against human H3K9me3S10p. The antigen used was a peptide containing the histone modification of interest. By plotting the absorbance against the antibody dilution (Figure 2), the titer of the antibody was estimated to be 1:87,000.



HeLa cells were stained with the antibody against H3K9me3S10p and with DAPI. Cells were fixed with 4% formaldehyde for 10' and blocked with PBS/TX-100 containing 5% normal goat serum and 1% BSA. The cells were immunofluorescently labelled with the H3K9me3S10p antibody (left) diluted 1:500 in blocking solution followed by an anti-rabbit antibody conjugated to Alexa488. The middle panel shows staining of the nuclei with DAPI. A merge of the two stainings is shown on the right.



A Dot Blot analysis was performed to test the cross reactivity of the antibody against H3K9me3S10p with peptides containing other modifications and unmodified sequences of histone H3. One hundred to 0.2 pmol of the peptide containing the respective histone modification were spotted on a membrane. The antibody was used at a dilution of 1:1,000. Image shows a high specificity of the antibody for the modification of interest.



ChIP using HeLa cells treated with colcemid (sheared chromatin from 10,000 cells per IP). Titration of 1, 5, and 10 µl ab per ChIP were analysed. Additionally, the same titration was analyzed after incubation with 5nM blocking peptide for 1hr at RT. IgG (5 µg/IP) was used as negative control. qPCR primers were for the promoter of GAPDH and c-fos and for the heterochromatin marker Sat2. Image shows the recovery, expressed as a % of input (the relative amount of IP'd DNA compared to input DNA after qPCR).