

Product datasheet for **TA347195**

H3FA (HIST1H3A) Rabbit Polyclonal Antibody

Product data:

Product Type:	Primary Antibodies
Applications:	Dot, ELISA, WB
Recommended Dilution:	ChIP/ChIP-seq (1-2 μ g/ChIP); ELISA (1:200); Dot blotting (1:2,000); Western blotting (1:1,000)
Reactivity:	Human
Host:	Rabbit
Isotype:	IgG
Clonality:	Polyclonal
Immunogen:	The immunogen for anti-H3K27me3S28p antibody: against histone H3, trimethylated at lysine 27 and phosphorylated at serine 28 (H3K27me3S28p), 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
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.



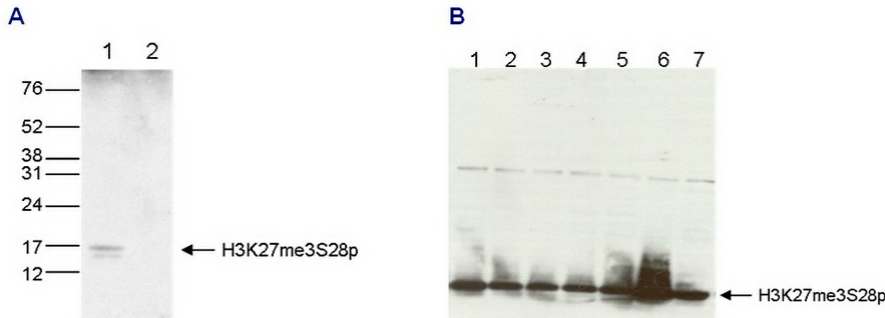
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Synonyms: A; H3; H3FA

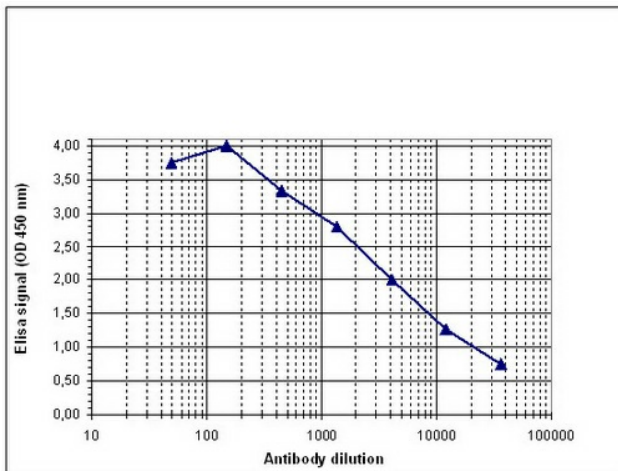
Protein Pathways: Systemic lupus erythematosus

Product images:

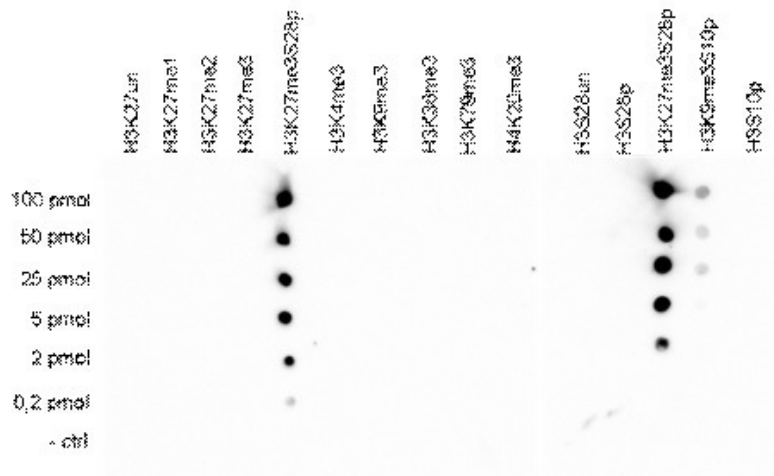
Figure 5



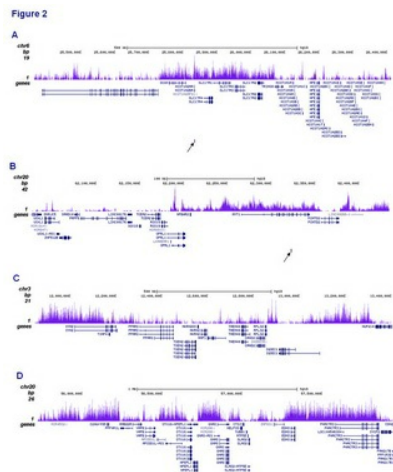
WB was performed on histone extracts (15 ug, lane 1) from HeLa cells, and on 1 ug of recombinant histone H3 (lane 2) using the ab at 1:100. B. Cell cycle experiment using the ab against H3K27me3S28p. WB was performed on cells blocked in different stages of the cell cycle (1=asynchronous population, 2=G1/Sa, 3=G1/Sb, 4=S phase, 5=G2/M, 6=M, 7=G1). The picture shows a nice peak of H3K27me3S28p expression during mitosis.



Determination of the antibody titer To determine the titer of the antibody, an ELISA was performed using a serial dilution of the antibody against H3K27me3S28p. The antigen used was a peptide containing the histone modification of interest. By plotting the absorbance against the antibody dilution (Figure 3), the titer of the antibody was estimated to be 1:4, 300.



A Dot Blot analysis was performed to test the cross reactivity of the antibody against H3K27me3S28p with peptides containing other modifications of histone H3 and H4 and the unmodified H3K27S28 sequence. 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 1:2,000. Figure 4 shows a high specificity of the antibody for the modification of interest.



ChIP was performed on sheared chromatin from 1.5 million colcemid treated HeLaS3 cells using 2 ug of the ab against H3K27me3S28p as described above. The IP'd DNA was subsequently analysed on an Illumina HiSeq. The 51 bp tags were aligned to the human genome using the BWA algorithm. Image shows the enrichment in genomic regions surrounding the TSH2B and MYT1 positive control genes (fig 2A and B, indicated by an arrow), and of chromosome 3 and 20 (figure 2C and D).