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Product datasheet for TA347179

H3FA (HIST1H3A) Rabbit Polyclonal Antibody

Product data:

Product Type:	Primary Antibodies
Applications:	Dot, ELISA, IF, WB
Recommended Dilution:	ChIP/ChIP-seq (1-2ug/IP); ELISA (1:1,000); Dot blotting (1:20,000); WB (1:1,000); IF (1:500)
Reactivity:	Human
Host:	Rabbit
Isotype:	IgG
Clonality:	Polyclonal
Immunogen:	The immunogen for anti-H3K9ac antibody: histone H3, acetylated at lysine 9 (H3K9ac), 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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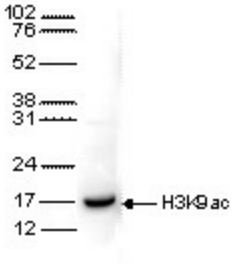
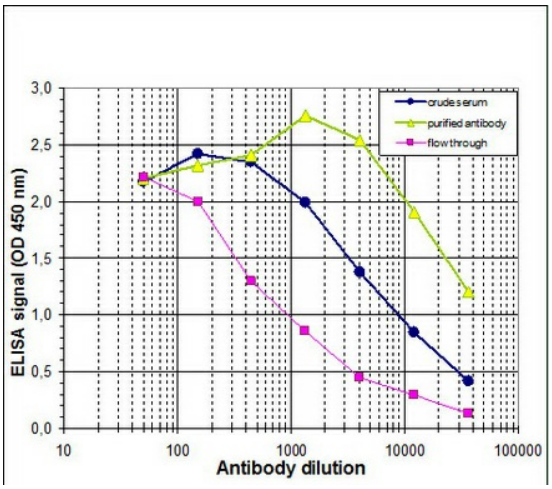
Synonyms: A; H3; H3FA

Protein Pathways: Systemic lupus erythematosus

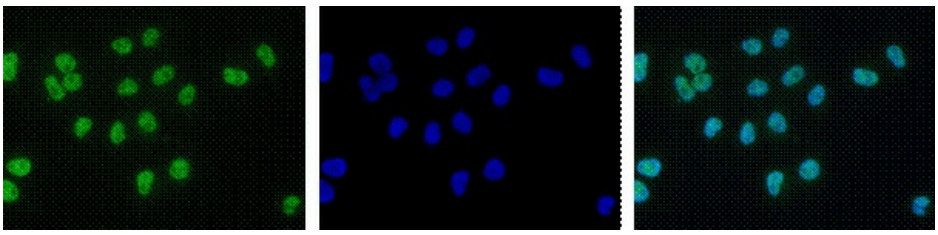
Product images:



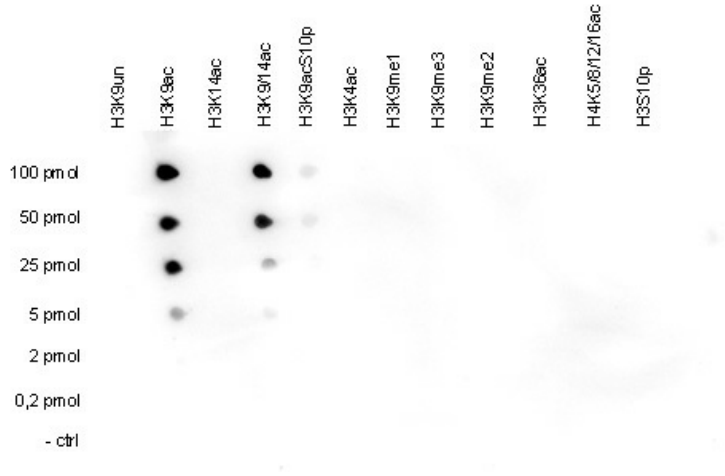
WB using the antibody against H3K9ac diluted 1:1,000 in TBS-Tween containing 5% skimmed milk. The position of the protein of interest is indicated on the right; the marker (in kDa) is shown on the left.



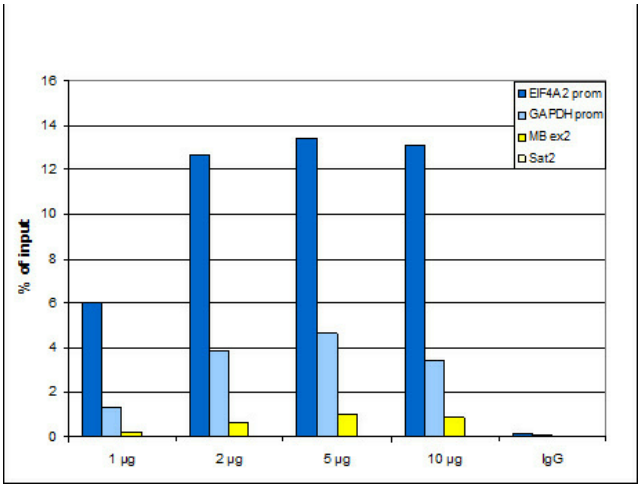
Determination of the antibody titer To determine the titer of the antibody, an ELISA was performed using a serial dilution of the antibody against H3K9ac in antigen coated wells. The antigen used was a peptide containing the histone modification of interest. By plotting the absorbance against the antibody dilution (Figure 3), the titer of the antibody was estimated to be 1:31, 700.



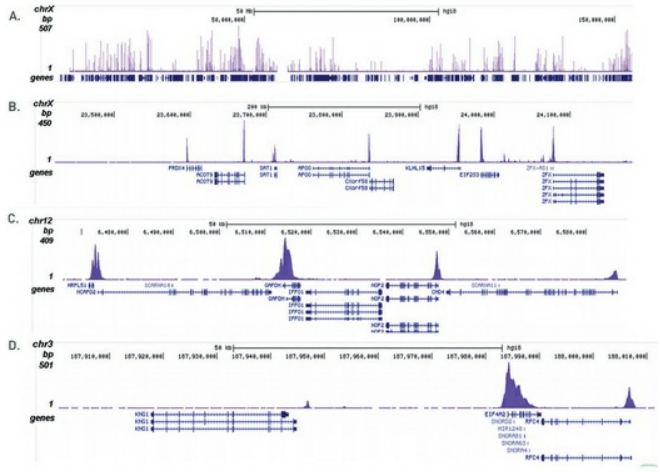
HeLa cells were stained with the antibody against H3K9ac and with DAPI. Cells were fixed with 4% formaldehyde for 10' and blocked with PBS/TX-100 containing 5% normal goat serum and 1% BSA. The cells were immunofluorescently labelled with the H3K9ac antibody (left) diluted 1:500 in blocking solution 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.



Dot Blot was performed to test the cross reactivity of the ab against H3K9ac with peptides containing other histone modifications and the unmodified H3K9 sequence. One hundred to 0.2 pmol of the respective peptides were spotted on a membrane. The antibody was used at a dilution of 1:20,000. Image shows a high specificity of the antibody for the modification of interest. Please note that this antibody recognizes the H3K9 acetylation, both in the presence and the absence of the K14 acetylation.



ChIP assays using HeLa cells (sheared chromatin from 1 million cells). Titration of 1, 2, 5 and 10 ug ab was used. IgG (2 ug/IP) was negative control. qPCR primers were for the promoter of GAPDH and EIF4A2as positive controls, and for exon 2 of the inactive MB gene and the Sat2 satellite repeat as negative controls. Image shows the recovery, expressed as a % of input (the relative amount of IP'd DNA compared to input DNA after qPCR). Results are in accordance with that acetylation of K9 at histone H3 is associated with the promoters of active genes.



ChIP was performed with 1 ug of the ab against H3K9ac and the IP'd DNA was subsequently analysed on an Illumina Genome Analyzer. The 36 bp tags were aligned to the human genome using the ELAND algorithm. Image shows the peak distribution along the complete sequence and an 800 kb region of the X-chromosome and in 100 kb regions surrounding the GAPDH and EIF4A2 positive control genes (figure 2C and D). These results clearly show an enrichment of H3K9ac at the promoters of active genes.