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Product datasheet for TA347180

H3FA (HIST1H3A) Rabbit Polyclonal Antibody

Product data:

Product Type:	Primary Antibodies
Applications:	Dot, ELISA, IF, WB
Recommended Dilution:	ChIP/ChIP-seq (1-2 ug/IP) ; ELISA (1:100); Dot blotting (1:20,000); Western blotting (1:1,000); Immunofluoresence (1:500)
Reactivity:	Human, Mouse
Host:	Rabbit
Isotype:	IgG
Clonality:	Polyclonal
Immunogen:	The immunogen for anti-H3K9/14ac antibody: histone H3 acetylated at lysines 9 and 14 (H3K9/14ac), 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 360198 MouseEntrez Gene 8350 Human P68431

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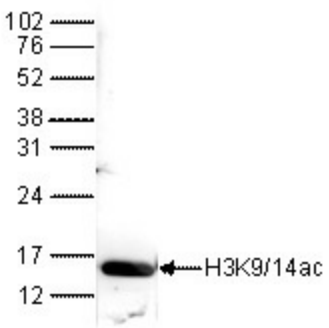
Background: Histones are the main constituents of the protein part of chromosomes of eukaryotic cells. They are rich in the amino acids arginine and lysine and have been greatly conserved during evolution. Histones pack the DNA into tight masses of chromatin. Two core histones of each class H2A, H2B, 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. Acetylation of H3K9/14 is enriched near the promoters of active genes.

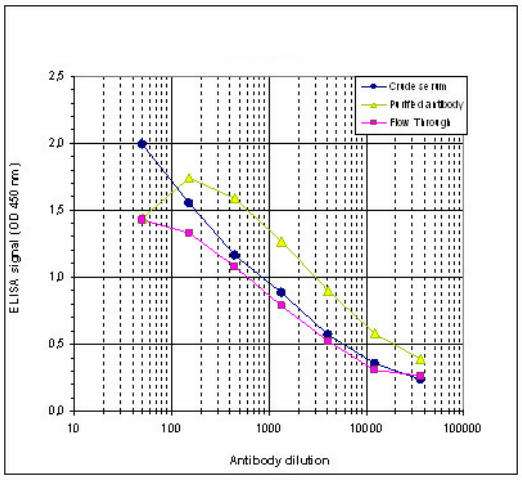
Synonyms: A; H3; H3FA

Protein Pathways: Systemic lupus erythematosus

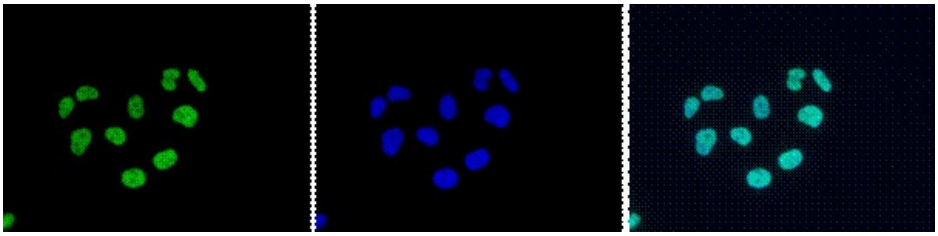
Product images:



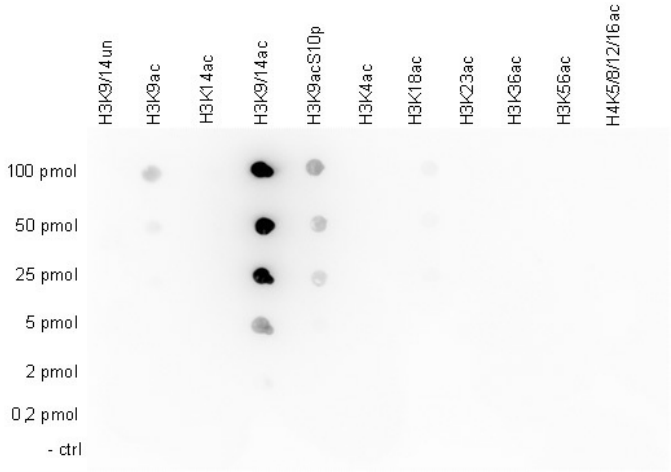
WB using the antibody against H3K9/14ac diluted 1:1,000 in TBS-Tween containing 5% skimmed milk. The position of the protein of interest is indicated on the right; the marker (in kDa) is shown on the left.



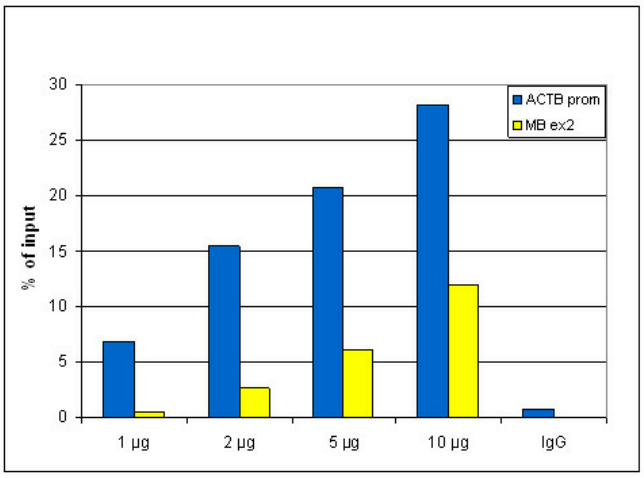
Determination of the antibody titer To determine the titer of the antibody, an ELISA was performed using a serial dilution of the antibody against H3K9/14ac, crude serum and flow through in antigen coated wells. The antigen used was a peptide containing the histone modification of interest. By plotting the absorbance against the antibody dilution (Figure 3), the titer of the purified antibody was estimated to be 1:5, 900.



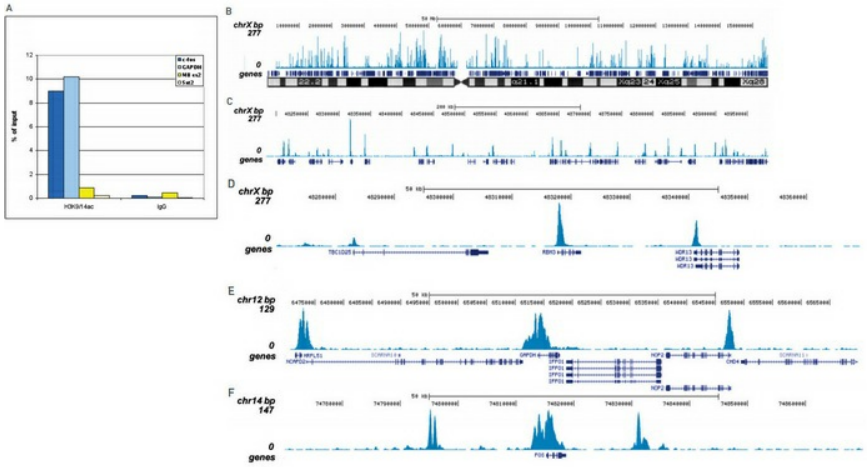
INIH3T3 cells were stained with the ab against H3K9/14ac and with DAPI. Cells were fixed with 4% formaldehyde for 10' and blocked with PBS/TX-100 containing 5% normal goat serum and 1% BSA. The cells were immunofluorescently labelled with the H3K9/14ac antibody (left) diluted 1:500 in blocking solution 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 H3K9/14ac with peptides containing other histone modifications and the unmodified H3K9/14 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. Figure 4 shows a high specificity of the antibody for the modification of interest



ChIP assays using HeLa cells (sheared chromatin from 1.5 million cells). Titration of 1, 2, 5 and 10ug ab per ChIP was analysed. IgG (5 ug/IP) was used as negative control. qPCR primers were for the promoter of ACTB as positive control and for exon 2 of MB as negative control. Image shows the recovery (the relative amount of IP'd DNA compared to input DNA). These results confirm the observation that acetylation of H3K9/14 is present at active promoters.



ChIP using 1 ug ab on sheared chromatin from 1 million HeLaS3 cells. IgG (2 ug/IP) was negative control. The IP'd DNA was analysed qPCR primers for the promoters of the active GAPDH and c-fos genes, used as positive control targets, and the coding region of inactive MB gene and the Sat2 satellite repeat, used as negative control targets and in 100 kb regions surrounding RBM3, GAPDH and c-fos genes (D, E and F). Results clearly show an enrichment of the H3K9/14 double acetylation at the promoters of active genes.