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Product datasheet for TA347204

H3FA (HIST1H3A) Rabbit Polyclonal Antibody

Product data:

Product Type:	Primary Antibodies
Applications:	Dot, ELISA
Recommended Dilution:	ChIP/ChIP-seq (5ug/ChIP); ELISA (1:500); Western blotting (1:20,000)
Reactivity:	Human
Host:	Rabbit
Isotype:	IgG
Clonality:	Polyclonal
Immunogen:	The immunogen for anti-H3K56ac antibody: the region of histone H3 containing the acetylated lysine 56 (H3K56ac), 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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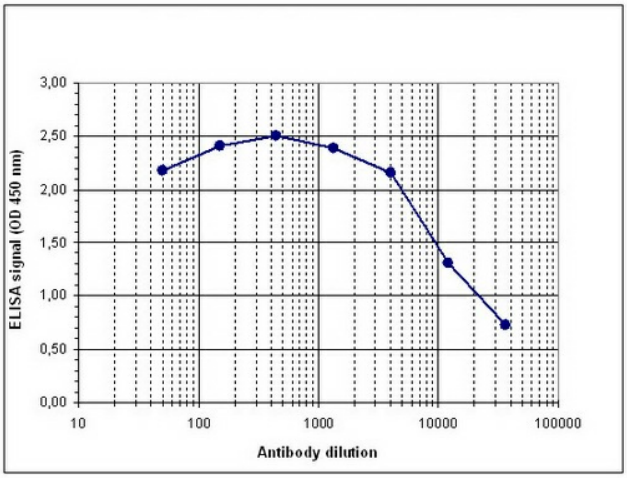
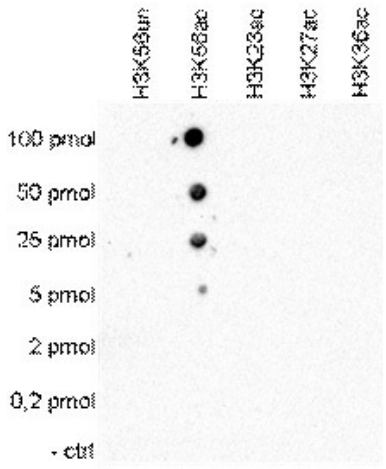
Synonyms: A; H3; H3FA

Protein Pathways: Systemic lupus erythematosus

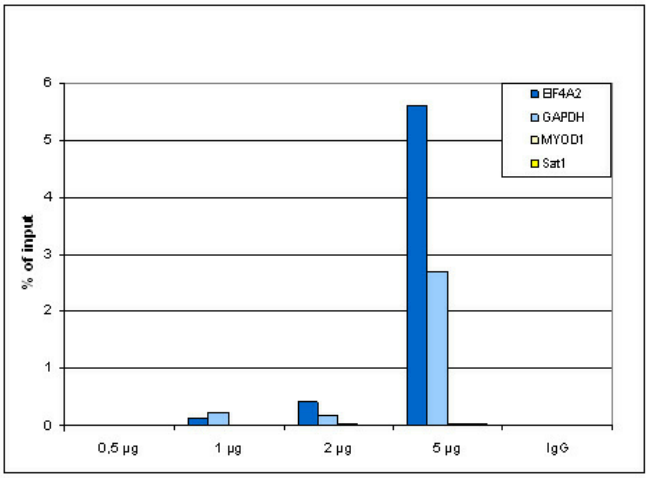
Product images:



Determination of the antibody titer To determine the titer of the antibody, an ELISA was performed using a serial dilution of the antibody against H3K56ac 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 1:15, 300.

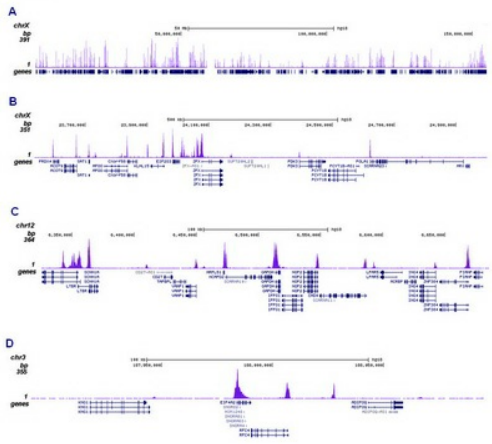


A Dot Blot analysis was performed with peptides containing other histone modifications and the unmodified H3K56. One hundred to 0.2 pmol of the respective peptides were spotted on a membrane. The antibody was used at a dilution of 1:20,000. Figure 4 shows a high specificity of the antibody for the modification of interest.



ChIP assays using HeLa cells (sheared chromatin from 1.5 million cells). Titration of 0.5, 1, 2 and 5ug antibody per ChIP was used. IgG (1 ug/IP) as negative control. qPCR primers were for a region ~1kb upstream of GAPDH promoter and for EIF4A2 promoter as positive controls, and for the coding region of inactive MYOD1 gene and 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).

Figure 2



ChIP was performed on sheared chromatin from 1.5 million HeLaS3 cells using 5 ug of the ab against H3K56ac 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 along the complete sequence and a 1 Mb region of the X-chromosome (fig 2A and B) and in genomic regions of chromosome 12 and 3, surrounding the GAPDH and EIF4A2 positive control genes.