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Product datasheet for TA347188

H3FA (HIST1H3A) Rabbit Polyclonal Antibody

Product data:

Product Type:	Primary Antibodies
Applications:	Dot, ELISA, WB
Recommended Dilution:	ChIP/ChIP-seq (1-2 ug/ChIP); ELISA (1:500); Dot blotting (1:20,000); Western blotting (1:1,000)
Reactivity:	Human
Host:	Rabbit
Isotype:	IgG
Clonality:	Polyclonal
Immunogen:	The immunogen for anti-H3K36me3 antibody: histone H3, trimethylated at lysine 36 (H3K36me3), 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. Trimethylation of H3K36 is associated with actively transcribed regions.

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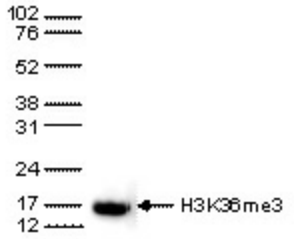
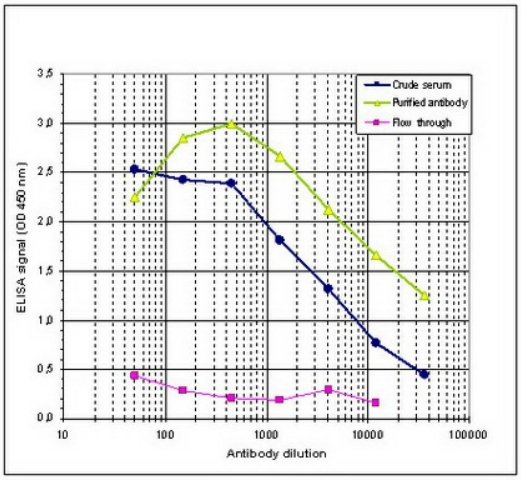
Synonyms: A; H3; H3FA

Protein Pathways: Systemic lupus erythematosus

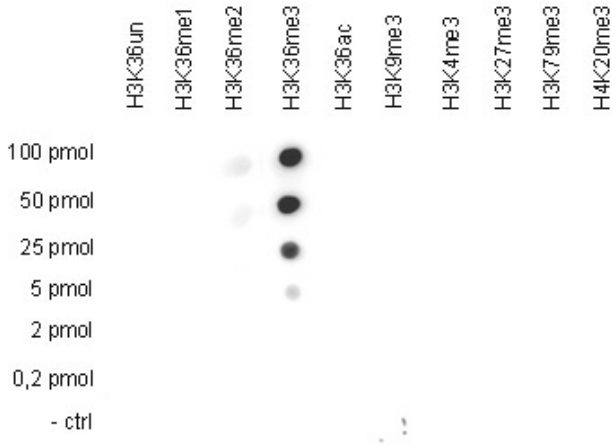
Product images:



WB using the antibody against H3K36me3 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 H3K36me3 and the crude serum. 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 1:19, 300.



A Dot Blot analysis was performed to test the cross reactivity of the antibody against H3K36me3 with peptides containing other H3 and H4 modifications and the unmodified sequence. One hundred to 0.2 pmol of peptide containing the respective histone modification 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.

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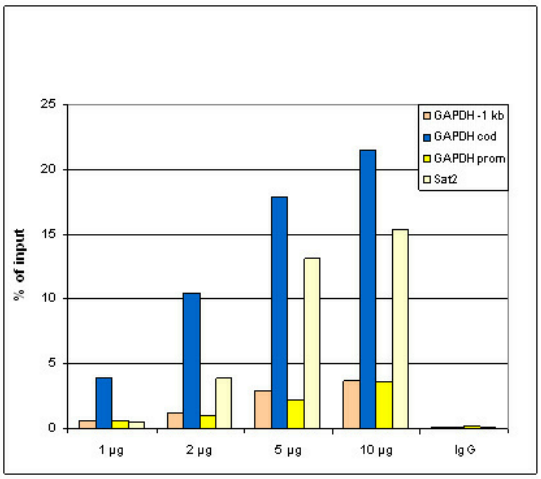
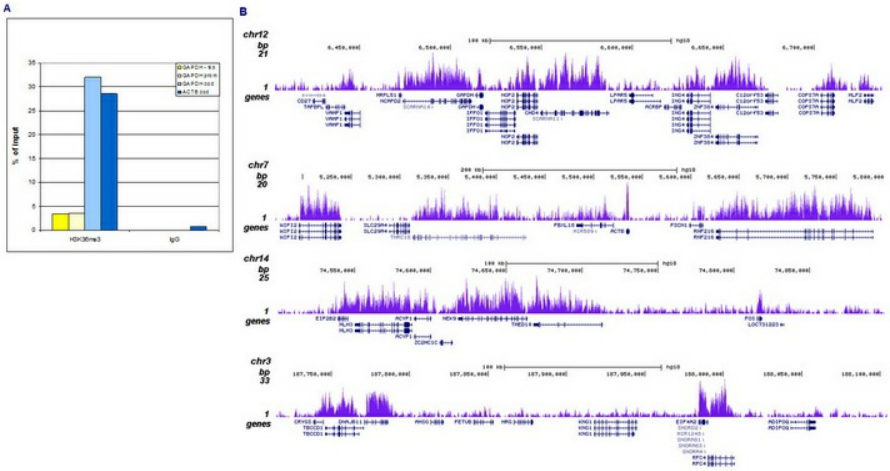


ChIP assays using HeLa cells (sheared chromatin from 1 million cells). Titration of 1, 2, 5 and 10 ug ab was used. IgG (2 ug/IP) was negative control. qPCR primers were for the promoter and coding region of the active GAPDH, for a region located 1 kb upstream of the GAPDH promoter and for the Sat2 satellite repeat. Image shows the recovery, expressed as a % of input (the relative amount of IP'd DNA compared to input DNA after qPCR).



ChIP using 2 ug ab on sheared chromatin from 1 million HeLaS3 cells. IgG (2 ug/IP) was negative control. The IP'd DNA was analysed with qPCR primers for the promoter and coding region of GAPDH, for a region 1 kb upstream of GAPDH promoter and for the coding region of active ACTB (Image shows the profiles in genomic regions of chromosome 12 (including GAPDH positive control), 7 (including ACTB positive control), 14 and 3, respectively. Results show an enrichment of the H3K36me3 at active genes.

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