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Product datasheet for TA347182

H3FA (HIST1H3A) Rabbit Polyclonal Antibody

Product data:

Product Type:	Primary Antibodies
Applications:	Dot, ELISA, IF, WB
Recommended Dilution:	ChIP/ChIP-seq (5 ug/ChIP) ; ELISA (1:500); Dot blotting (1:20,000); Western blotting (1:1,000); IF (1:5,000)
Reactivity:	Human
Host:	Rabbit
Isotype:	IgG
Clonality:	Polyclonal
Immunogen:	The immunogen for anti-H3K4me2 antibody: histone H3 containing the dimethylated lysine 4 (H3K4me2), 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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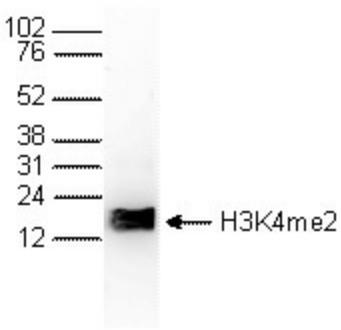
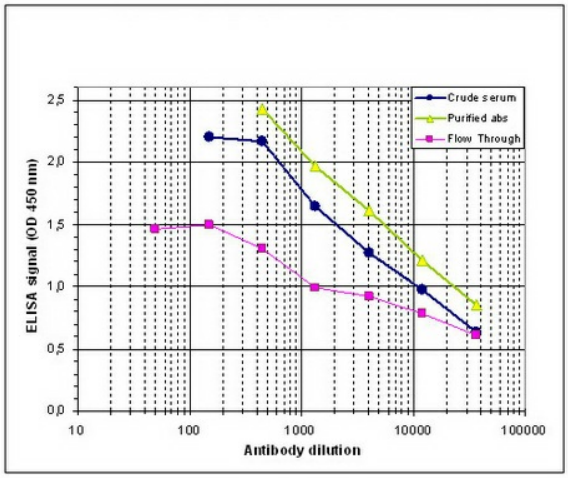
Synonyms: A; H3; H3FA

Protein Pathways: Systemic lupus erythematosus

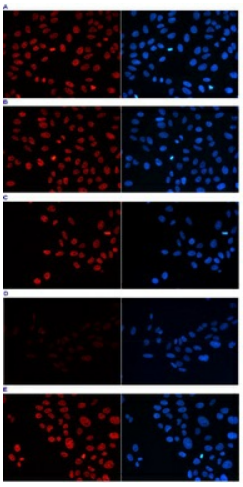
Product images:



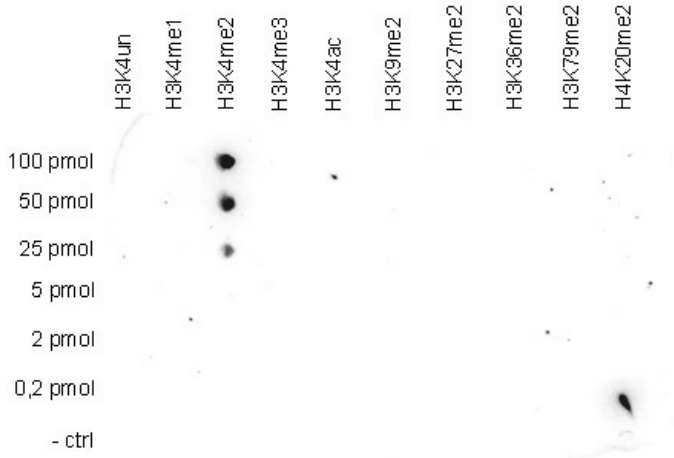
WB using the antibody against H3K4me2 diluted 1:1,000 in TBS-Tween containing 5% skimmed milk. The position of the protein of interest is indicated on the right; the marker (in kDa) is shown on the left.



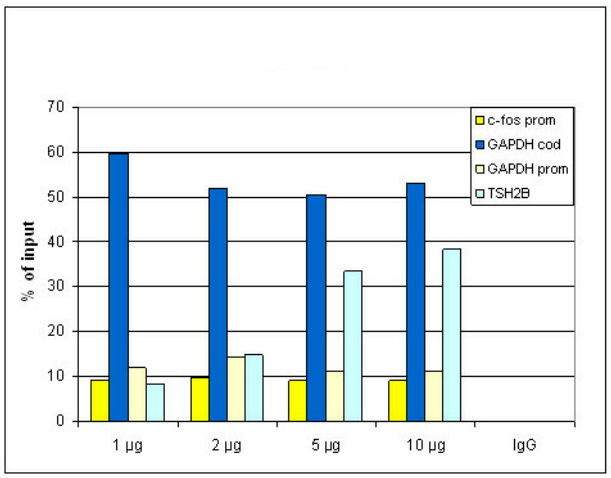
Determination of the titer To determine the titer, an ELISA was performed using a serial dilution of the antibody against H3K4me2, crude serum and flow through in antigen coated wells. The antigen used was a peptide containing the histone modification of interest. By plotting the absorbance against the antibody dilution (Figure 3), the titer of the antibody was estimated to be 1:12, 600.



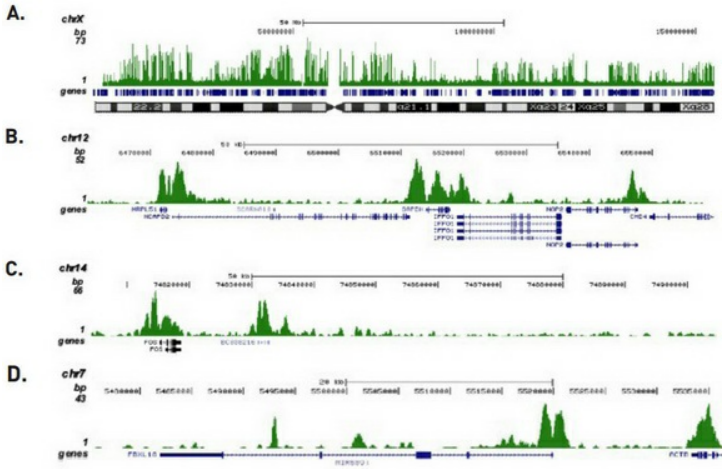
Human U2OS cells were fixed with 4% formaldehyde and blocked with PBS/TX-100 containing 5% normal goat serum. A: cells were labeled with the H3K4me2 antibody (left) at 1:5,000 in blocking solution followed by an anti-rabbit conjugated to Alexa568 or with DAPI (right). B, C, D and E: staining of the cells with the H3K4me2 antibody after incubation of the antibody with 5 ng/ul blocking peptide containing the unmodified and the mono-, di- and trimethylated H3K4, respectively.



A Dot Blot analysis was performed to test the cross reactivity of the antibody against H3K4me2 with peptides containing other modifications of histone H3 and H4 and the unmodified H3K4 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 was performed with the ab against H3K4me2 on sheared chromatin from 1 million HeLaS3 cells using the “Auto Histone ChIP experiment was analysed. IgG (2 ug/IP) was used as negative control. qPCR primers were for the promoter and coding region of the active GAPDH gene, the promoter of the active c-fos gene and for the coding region of the inactive TSH2B. Image shows the recovery, expressed as a % of input (the relative amount of IP'd DNA compared to input DNA after qPCR analysis).



ChIP was performed as described above using 1 ug of the ab against H3K4me2 (cat. No. pAb-035-050). The IP'd DNA was 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 X-chromosome and in 3 chromosomal regions surrounding the GAPDH, c-fos and ACTB genes (figure 2B, C and D, respectively).