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Product datasheet for TA347183

H3FA (HIST1H3A) Rabbit Polyclonal Antibody

Product data:

Product Type:	Primary Antibodies
Applications:	Dot, ELISA, WB
Recommended Dilution:	ChIP/ChIP-seq (1-2 µg/ChIP) ; ELISA (1:500); Dot blotting (1:10,000); Western blotting (1:500)
Reactivity:	Human
Host:	Rabbit
Isotype:	IgG
Clonality:	Polyclonal
Immunogen:	The immunogen for anti-H3K4me1 antibody: histone H3 containing the monomethylated lysine 4 (H3K4me1), 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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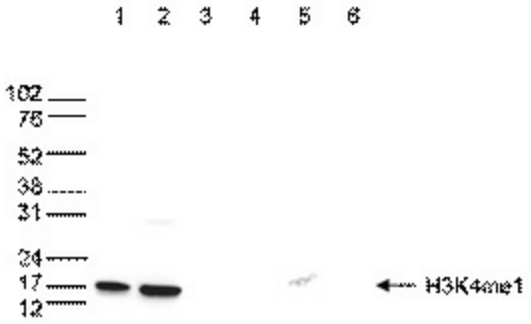
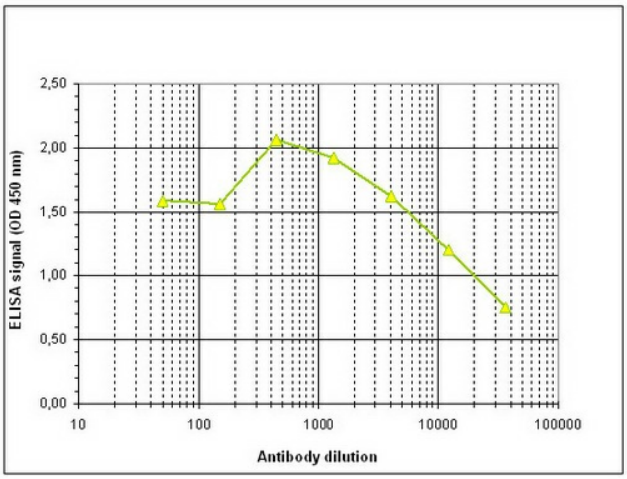
Synonyms: A; H3; H3FA

Protein Pathways: Systemic lupus erythematosus

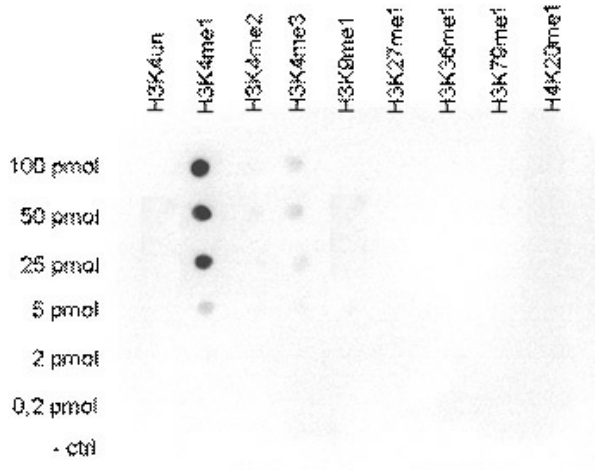
Product images:



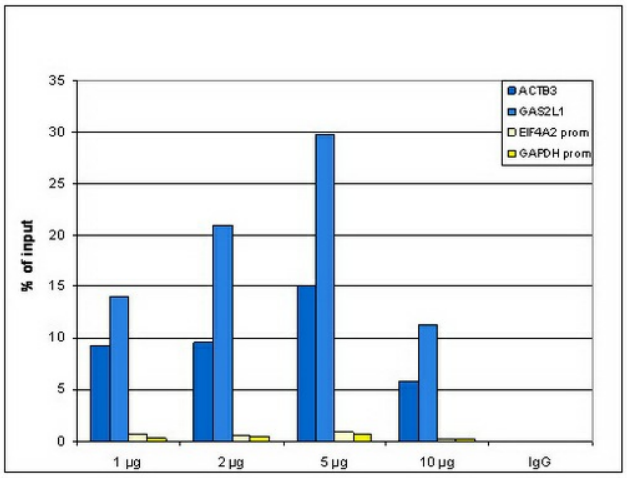
WB was performed on whole cell (25 ug, lane 1) and histone extracts (15 ug, lane 2) from HeLa cells, and on 1 ug of recombinant histone H2A, H2B, H3 and H4 (lane 3, 4, 5 and 6, respectively) using the antibody against H3K4me1. The antibody was diluted 1:500 in TBS-Tween containing 5% skimmed milk. The position of the protein of interest is shown on the right, the marker (in kDa) is shown on the left.



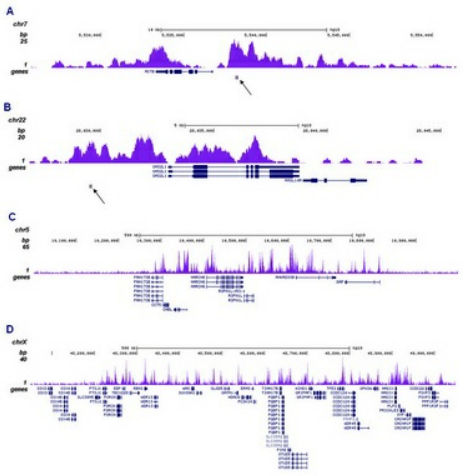
Determination of the titer To determine the titer, an ELISA was performed using a serial dilution of the antibody against H3K4me1 in antigen coated wells. The antigen used was a peptide containing the histone modification of interest. By plotting the absorbance against the antibody dilution (Figure 3), the titer of the antibody was estimated to be 1:20, 100.



A Dot Blot analysis was performed to test the cross reactivity of the antibody against H3K4me1 with peptides containing other modifications or unmodified sequences of histone H3. One hundred to 0.2 pmol of the respective peptides were spotted on a membrane. The antibody was used at a dilution of 1:10,000. Figure 4 shows a high specificity of the antibody for the modification of interest.



ChIP was performed with the ab against H3K4me1 on sheared chromatin from 1 million HeLaS3 cells using the “iDeal ChIP experiment was analysed. IgG (2 ug/IP) was used as negative IP control. qPCR was performed with primers for a region surrounding the ACTB and GAS2L1 genesas positive controls, and for the promoters of the GAPDH and EIF4A2 genesas negative controls. 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 with 1 ug of the ab against H3K4me1. 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. Figure 2A and B show the enrichment in chromosomal regions surrounding the ACTB and GAS2L1 positive control genes. The position of the amplicon used in the qPCR validation is indicated by an arrow. Figure 2C and D show the H3K4me1 signal in two 1 Mb regions of chromosome 5 and X, respectively.