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Product datasheet for TA347181

H3FA (HIST1H3A) Rabbit Polyclonal Antibody

Product data:

Product Type:	Primary Antibodies
Applications:	Dot, IF, WB
Recommended Dilution:	ChIP (10 µg per IP); Dot blotting (1:20,000); Western blotting (1:500); IF (1:500)
Reactivity:	Human
Host:	Rabbit
Isotype:	IgG
Clonality:	Polyclonal
Immunogen:	The immunogen for anti-H3K4me3 antibody: the region of histone H3 containing the trimethylated lysine 4 (H3K4me3), 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 histone H3K4 is associated with active promoters.

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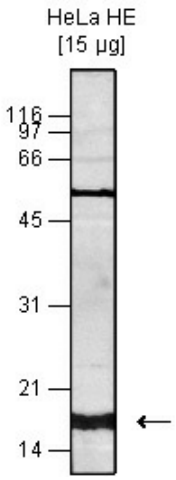
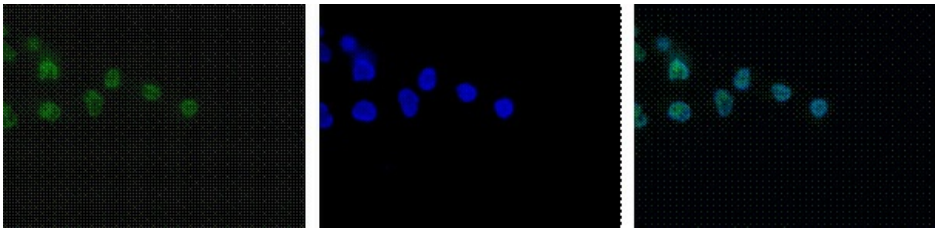
Synonyms: A; H3; H3FA

Protein Pathways: Systemic lupus erythematosus

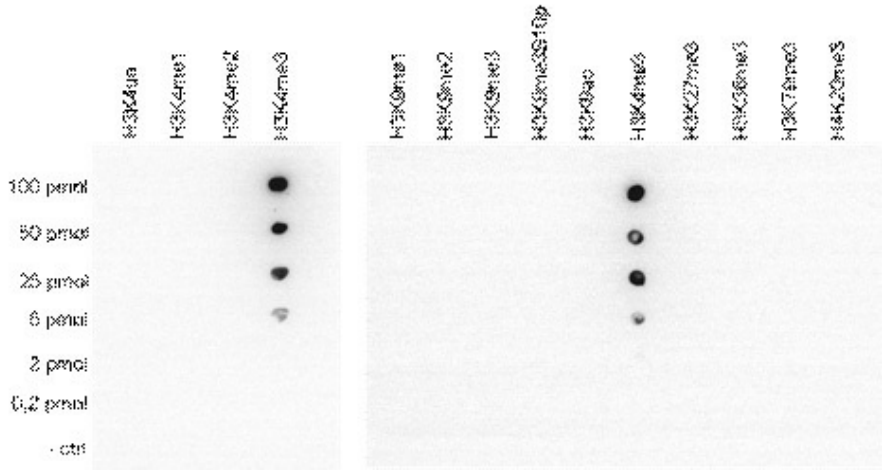
Product images:



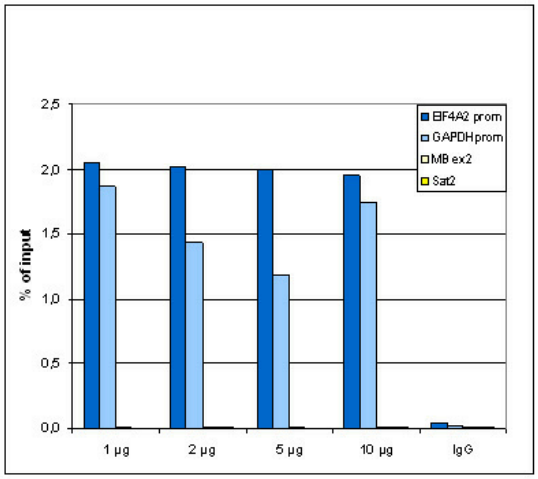
WB was performed using histone extracts from HeLa cells (HeLa HE, 15 ug) and the antibody against H3K4me3 diluted 1:500 in TBS-Tween containing 5% skimmed milk. A molecular weight marker (in kDa) is shown on the left, the position of the protein of interest is shown on the right.



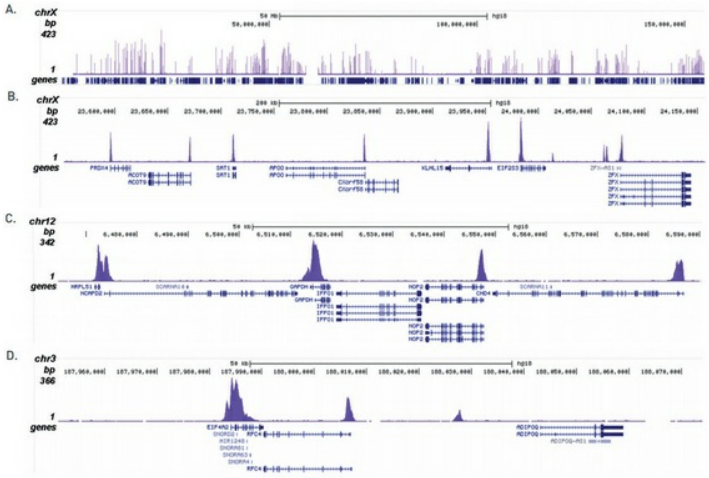
HeLa cells were stained with the antibody against H3K4me3 and with DAPI. Cells were fixed with 4% formaldehyde for 10¡¯ and blocked with PBS/TX-100 containing 5% normal goat serum and 1% BSA. The cells were immunofluorescently labelled with the H3K4me3 antibody (left) diluted 1:500 in blocking solution 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 H3K4me3 with peptides containing other modifications and unmodified sequences of histone H3 and H4. One hundred to 0.2 pmol of the respective peptides were spotted on a membrane. The antibody was used at a dilution of 1:2,000. Image shows a high specificity of the antibody for the modification of interest.



ChIP assays using HeLa cells (sheared chromatin from 1 million cells). Titration of 1, 2, 5 and 10ug ab was used. IgG (2 ug/IP) was negative control. qPCR primers were for the promoter of GAPDH and EIF4A2 as positive controls, and for exon 2 of inactive MB 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). These results are in accordance with that trimethylation of K4 at histone H3 is associated with the promoters of active genes



ChIP was performed on sheared chromatin from 1 million HeLa cells using 1 ug ab. 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. Image shows the peak distribution along the complete sequence and a 600 kb region of the X-chromosome and in two regions surrounding GAPDH and EIF4A2 positive control genes (C&D). These results show an enrichment of the H3K4 trimethylation at the promoters of active genes.