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Product datasheet for TA347192

H3FA (HIST1H3A) Rabbit Polyclonal Antibody

Product data:

Product Type:	Primary Antibodies
Applications:	Dot, ELISA, IF, WB
Recommended Dilution:	ChIP/ChIP-seq (1ug/ChIP); ELISA (1:200); Dot blotting (1:5,000); Western blotting (1:500); Immunofluoresence (1:200)
Reactivity:	Human
Host:	Rabbit
Isotype:	IgG
Clonality:	Polyclonal
Immunogen:	The immunogen for anti-H3K27me3 antibody: against histone H3, trimethylated at lysine 27 (H3K27me3), 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

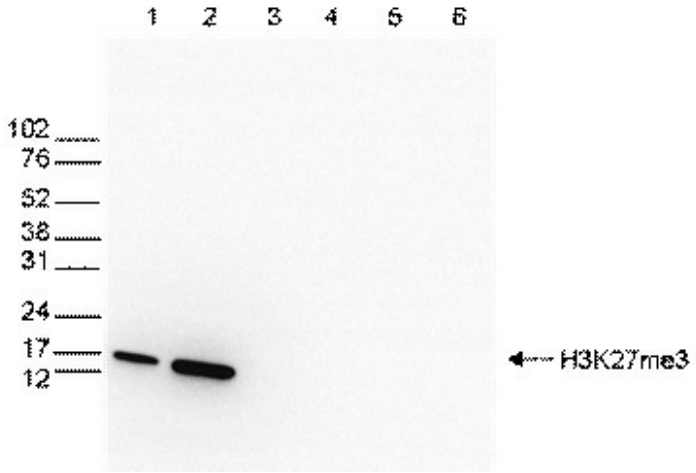
View online »

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 H3K27 is associated with gene repression.

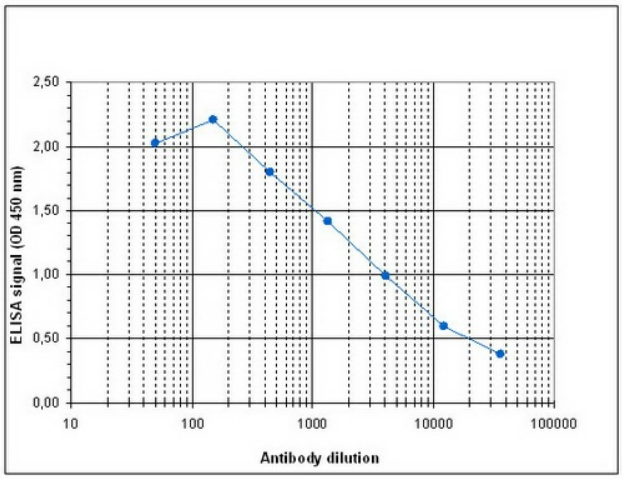
Synonyms: A; H3; H3FA

Protein Pathways: Systemic lupus erythematosus

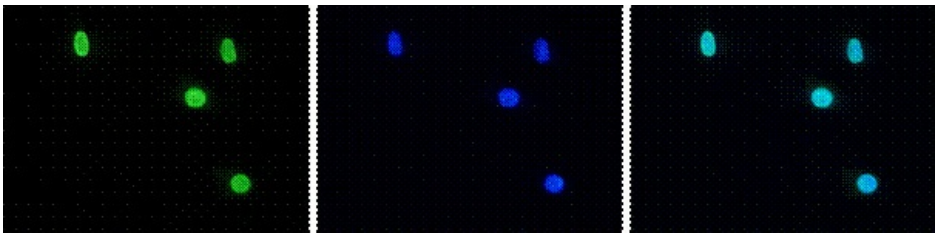
Product images:



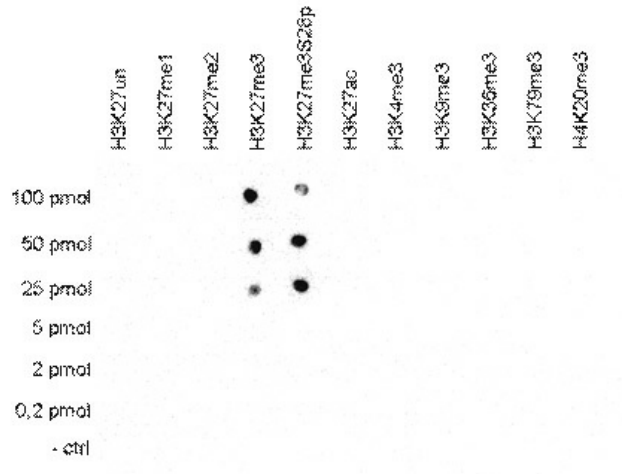
WB was performed on whole cell (25 ug, lane 1) and histone extracts (15 ug, lane 2) from HeLa cells, and on 1 ug of recombinant histone H2A, H2B, H3 and H4 (lane 3, 4, 5 and 6, respectively) using the antibody against H3K27me3 diluted 1:500 in TBS-Tween containing 5% skimmed milk. The position of the protein of interest is indicated on the right; the marker (in kDa) is shown on the left.



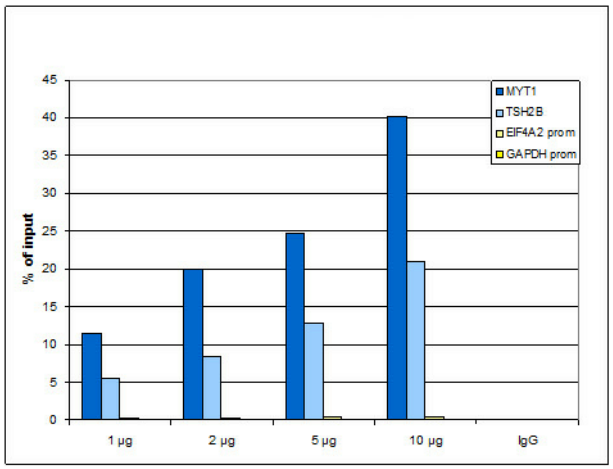
Determination of the antibody titer To determine the titer of the antibody, an ELISA was performed using a serial dilution of the antibody against H3K27me3. The antigen used was a peptide containing the histone modification of interest. By plotting the absorbance against the antibody dilution (Figure 3), the titer of the antibody was estimated to be 1:3, 500.



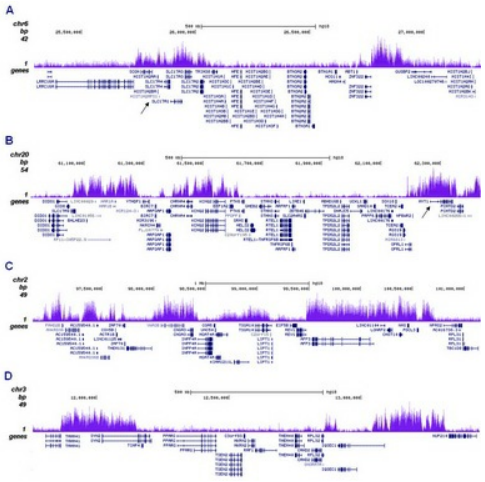
INIH3T3 cells were stained with the ab against H3K27me3 and with DAPI. Cells were fixed with 4% formaldehyde for 10' and blocked with PBS/TX-100 containing 5% normal goat serum and 1% BSA. The cells were immunofluorescently labelled with the H3K27me3 antibody (left) diluted 1:200 in blocking solution 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.



Dot Blot was performed to test the cross reactivity of the ab against H3K27me3 with peptides containing other modifications of histone H3 and H4 and the unmodified H3K27 sequence. One hundred to 0.2 pmol of the peptide containing the respective histone modification were spotted on a membrane. The antibody was used at 1:5,000. Image shows a high specificity of the antibody for the modification of interest. Please note that that antibody also recognizes the modification if S28 is phosphorylated.



ChIP using HeLa cells (1 million cells). Titration of 1, 2, 5 and 10ug ab per ChIP was used. IgG (2 ug/IP) as negative control. qPCR primers were for the promoters of active genes EIF4A2 and GAPDH as negative controls, and for the coding regions of inactive genes MYT1 and TSH2B as positive controls. Image shows the recovery, expressed as a % of input (the relative amount of IP'd DNA compared to input DNA after qPCR). Results show that H3K27me3 is preferably present at inactive genes.



ChIP was performed on sheared chromatin from 1 million HeLaS3 cells using 1 ug of the ab against H3K27me3 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 in genomic regions of chromosome 6, surrounding the TSH2B gene (indicated by an arrow; fig 2A), of chromosome 20, surrounding the MYT1 gene (fig 2B), and of chromosome 2 and 3 (figure 2C and D).