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Product datasheet for TA347216

H4-16 Rabbit Polyclonal Antibody

Product data:

Product Type:	Primary Antibodies
Applications:	Dot, ELISA, IF, WB
Recommended Dilution:	ChIP/ChIP-seq (1-2 µg/ChIP); ELISA (1:100); Dot blotting (1:20,000); Western blotting (1:1,000); Immunofluorescence (1:300)
Reactivity:	Human
Host:	Rabbit
Isotype:	IgG
Clonality:	Polyclonal
Immunogen:	The immunogen for anti-H4K20me3 antibody: the region of histone H4 containing the trimethylated lysine 20 (H4K20me3), 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 4, H4
Database Link:	NP_778224 Entrez Gene 121504 Human P62805
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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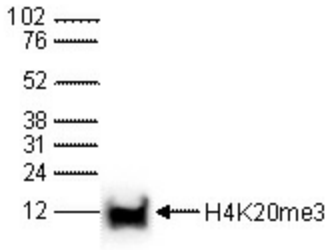
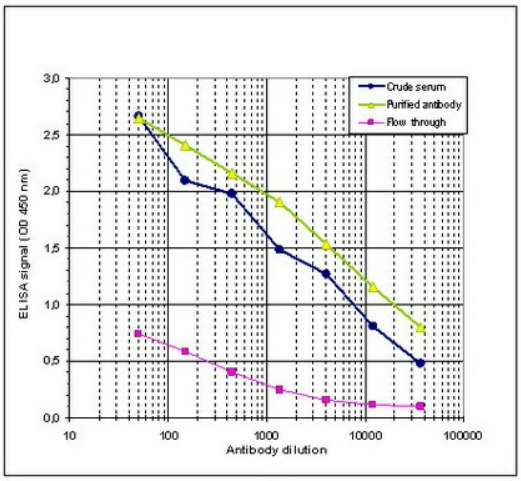
Synonyms: H4; p

Protein Pathways: Systemic lupus erythematosus

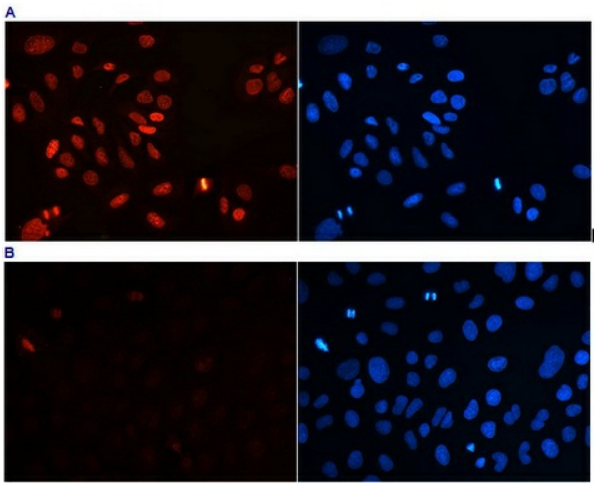
Product images:



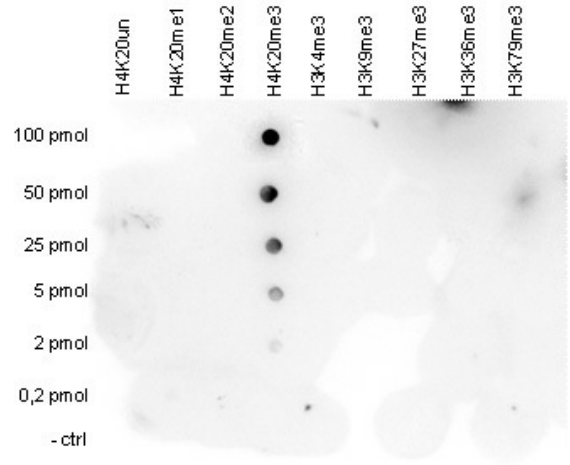
WB using the antibody against H4K20me3 diluted 1:1,000 in TBS-Tween containing 5% skimmed milk. The position of the protein of interest is indicated on the right; the marker (in kDa) is shown on the left.



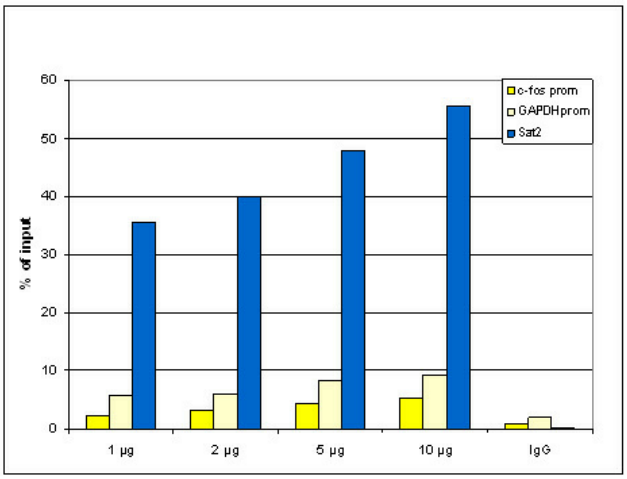
Determination of the antibody titer To determine the titer of the antibody, an ELISA was performed using a serial dilution of the antibody against H4K20me3, crude serum and flow through in antigen coated wells. The antigen used was a peptide containing the histone modification of interest. By plotting the absorbance against the antibody dilution (Figure 3), the titer of the antibody was estimated to be 1:7, 400.



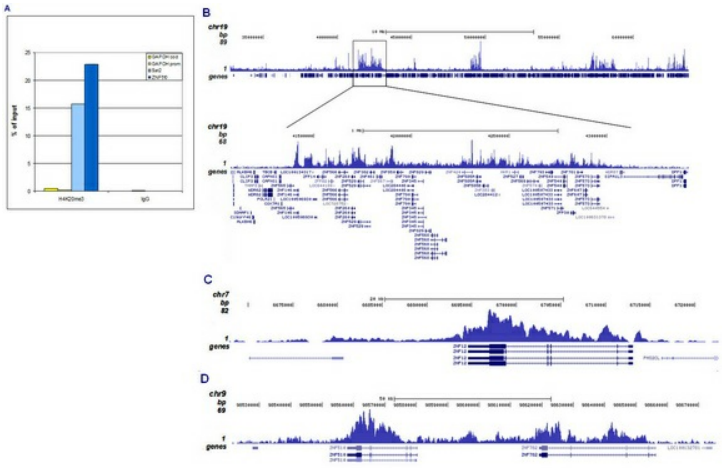
Human U2OS cells were fixed with ice cold methanol for 10' and blocked with PBS/TX-100 containing 5% normal goat serum. Figure 6A: cells were labeled with the H4K20me3 antibody (left) diluted 1:300 in blocking solution followed by an anti-rabbit antibody conjugated to Alexa568 or with DAPI (right), which specifically labels DNA. Figure 6B: staining of the cells with the H4K20me3 antibody after incubation of the antibody with blocking peptide (concentration: 5 ng/ul).



A Dot Blot analysis was performed to test the cross reactivity of the antibody against H4K20me3 with peptides containing other histone modifications and the unmodified H4K20. 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 assays using HeLa cells (sheared chromatin from 1 million cells). A titration of the antibody consisting of 1, 2, 5, and 10 ug per ChIP experiment was analysed. IgG (1 ug/IP) was used as negative control. qPCR primers were for promoters of the active genes c-fos and GAPDHas negative controls, and for the Sat2 satellite repeat region positive control. Image shows the recovery, expressed as a % of input (the relative amount of IP'd DNA compared to input DNA after qPCR).



ChIP using 1ug ab on sheared chromatin from 1 million HeLaS3 cells. The IP'd DNA was analysed with qPCR primers for the promoter and coding region of GAPDH, for the coding region of the ZNF510 and for the Sat2 satellite repeat (Image shows the signal distribution along the long arm of chromosome 19 and a zoomin to an enriched region containing several ZNF repeat genes. C and D show the enrichment at ZNF12 and ZNF510 on Chr 7 and 9. Results show an enrichment of H4K20me3 at ZNF repeat genes.