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Product datasheet for TA347212

H4-16 Rabbit Polyclonal Antibody

Product data:

Product Type:	Primary Antibodies
Applications:	Dot, ELISA, IF, WB
Recommended Dilution:	ChIP/ChIP-seq (0.5-1ug/IP); ELISA (1:1000); Dot blotting (1:20,000); Western blotting (1:1,000); Immunofluorescence (1:500)
Reactivity:	Human, Mouse, Broad
Host:	Rabbit
Isotype:	IgG
Clonality:	Polyclonal
Immunogen:	The immunogen for anti-H4K5,8,12ac antibody: the region of histone H4 containing the acetylated lysines 5, 8 and 12 (H4K5,8,12ac), 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 320332 MouseEntrez Gene 121504 Human P62805

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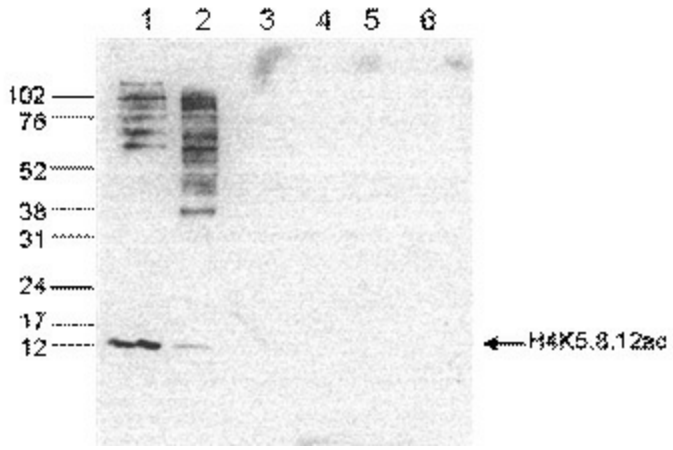
Background: Histones are the main constituents of the protein part of chromosomes of eukaryotic cells. They are rich in the amino acids arginine and lysine and have been greatly conserved during evolution. Histones pack the DNA into tight masses of chromatin. Two core histones of each class H2A, H2B, 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. Acetylation of histone H4 is associated with active genes.

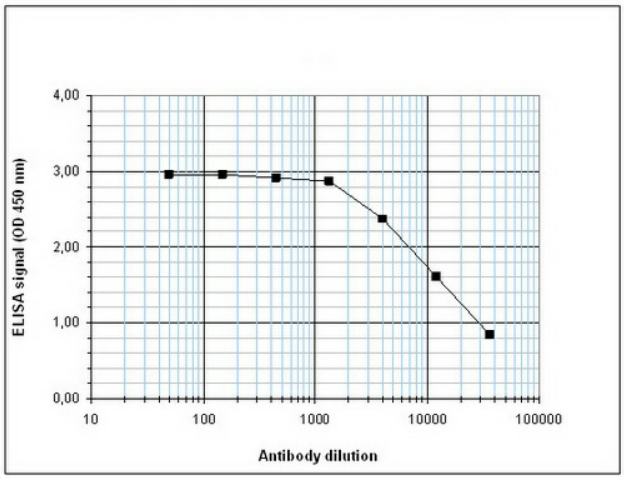
Synonyms: H4; p

Protein Pathways: Systemic lupus erythematosus

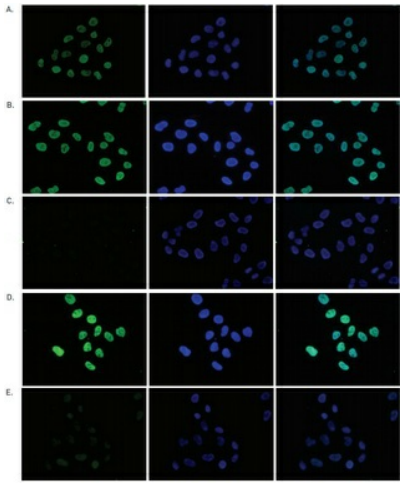
Product images:



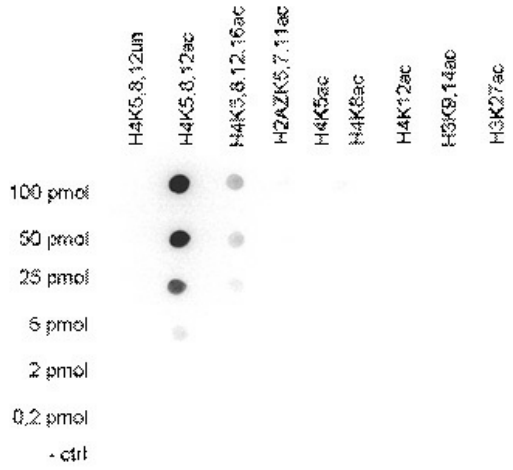
WB was performed on whole cell (25 ug, lane 1) and histone extracts (15 ug, lane 2) from HeLa cells, and on 1 ug of recombinant histone H2A, H2B, H3 and H4 (lane 3, 4, 5 and 6, respectively) using the antibody against H4K5, 8, 12ac. The antibody was diluted 1:1,000 in TBS-Tween containing 5% skimmed milk. The position of the protein of interest is indicated on the right, the marker (in kDa) is shown on the left.



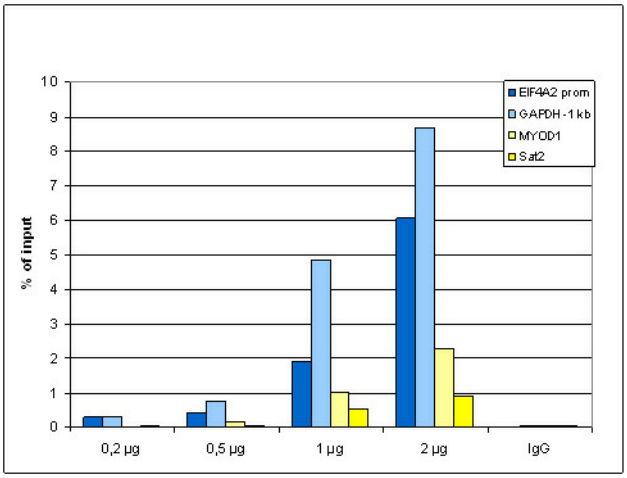
Determination of the antibody titer To determine the titer of the antibody, an ELISA was performed using a serial dilution of the antibody against H4K5, 8, 12ac in antigen coated wells. The antigen used was a peptide containing the histone modification of interest. By plotting the absorbance against the antibody dilution (Figure 3), the titer of the antibody was estimated to be 1:14, 500.



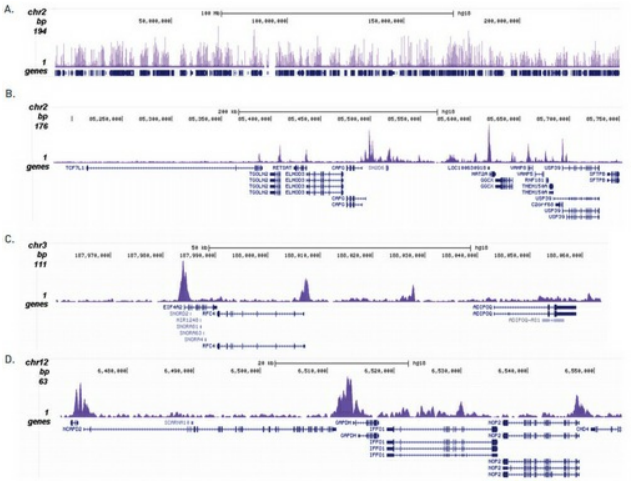
HeLa cells were fixed with 4% formaldehyde and blocked with PBS/TX-100 containing 5% normal goat serum and 1% BSA. Image shows staining of the nuclei with DAPI. A merge of the two stainings is shown on the right. B, C, D and E: staining of the cells with the H4K5, 8, 12ac antibody after incubation of the antibody with 10 ng/ul of the following blocking peptides: H4K5, 8, 12 unmodified (B), H4K5, 8, 12ac (C), H2A.ZK5, 7, 11ac (D) and H4K5, 8, 12, 16ac (E).



A Dot Blot analysis was performed with peptides containing other histone modifications and the unmodified H4. One hundred to 0.2 pmol of the respective peptides were spotted on a membrane. The antibody was used at a dilution of 1:20,000. Figure 4 shows a high specificity of the antibody for the modification of interest.



ChIP was performed with the "iDeal ChIP experiment was analysed. IgG (1 ug/IP) was used as negative IP control. QPCR was performed with primers for promoter of the active gene EIF4A2 and for a region 1 kb upstream of the GAPDH gene, used as positive controls, and for the inactive MYOD1 gene and the Sat2 satellite repeat region used as negative controls. Image shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).



ChIP was performed with 0.5 ug of the ab on sheared chromatin from 100,000 K562 cells. 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 signal distribution along the complete length of chromosome 2 and a zoomin to a 600 kb region (B). C and D show the enrichment in two genomic regions on chromosome 3 and 12, respectively, containing EIF4A2 and GAPDH positive controls.