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Product datasheet for TA347133

H2A.Z (H2AFZ) Rabbit Polyclonal Antibody

Product data:

Product Type:	Primary Antibodies
Applications:	Dot, ELISA, IF
Recommended Dilution:	ChIP/ChIP-seq (1ug per ChIP); ELISA (1:500 ?? 1:200); Dot blotting (1:20,000); Immunofluoresence (1:500)
Reactivity:	Human
Host:	Rabbit
Isotype:	IgG
Clonality:	Polyclonal
Immunogen:	The immunogen for anti-H2A.Zac antibody: histone H2A.Z acetylated at lysines 5, 7 and 11, 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:	H2A histone family member Z
Database Link:	NP_002097 Entrez Gene 3015 Human P0C0S5

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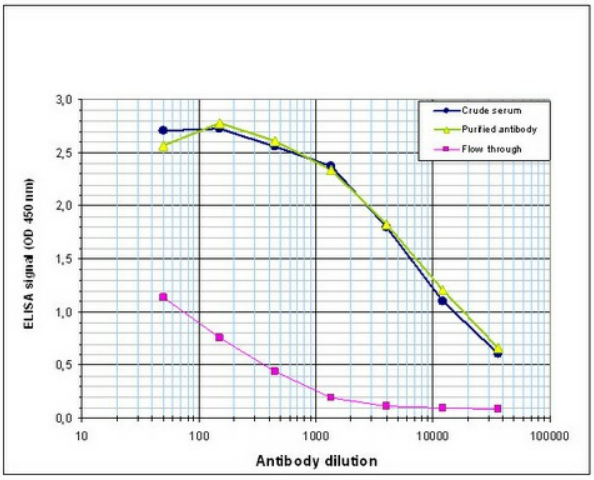
Background: Histones are the main constituents of the protein part of chromosomes of eukaryotic cells. They are rich in the amino acids arginine and lysine and have been greatly conserved during evolution. Histones pack the DNA into tight masses of chromatin. Two core histones of each class H2A, H2B,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

Synonyms: H2A; H2A.z; H2A.Z-1; H2AZ; z

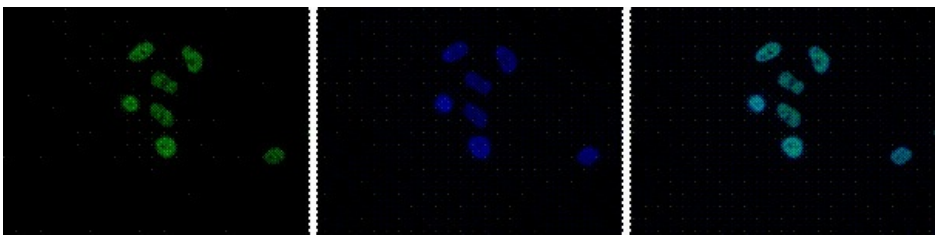
Protein Families: Druggable Genome

Protein Pathways: Systemic lupus erythematosus

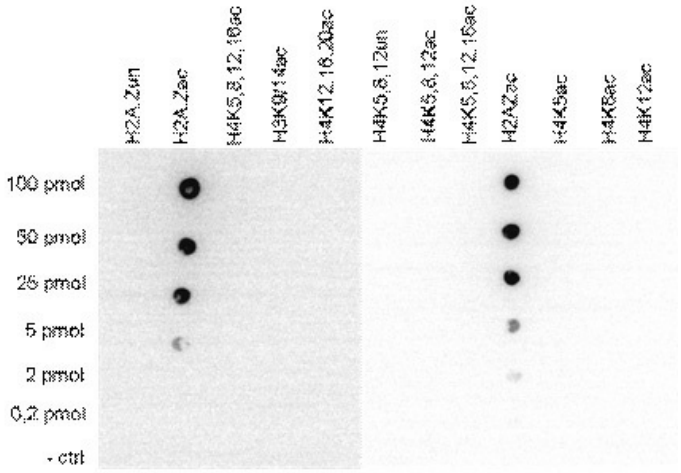
Product images:



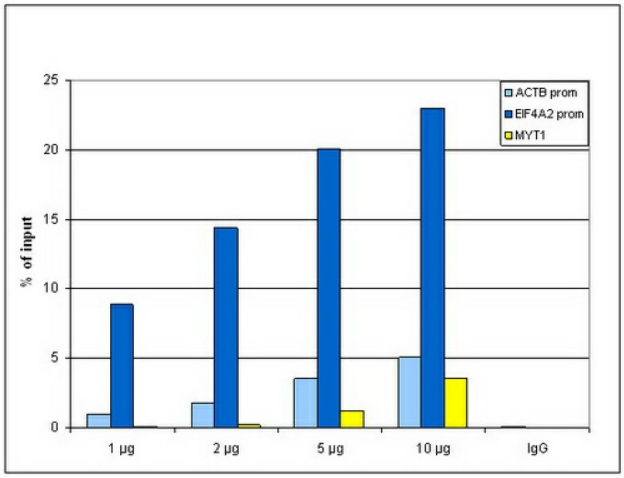
Determination of the antibody titer To determine the titer of the antibody, an ELISA was performed using a serial dilution of the antibody against H2A.Zac, crude serum and flow through in antigen coated wells. The antigen used was a peptide containing the histone modifications of interest. By plotting the absorbance against the antibody dilution (Figure 3), the titer of the purified antibody was estimated to be 1:8, 800.



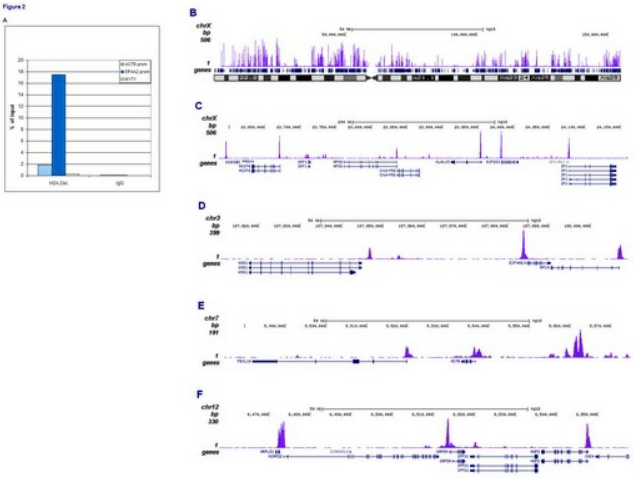
HeLa cells were stained with the antibody against H2A.Zac and with DAPI. Cells were fixed with 4% formaldehyde for 10' and blocked with PBS/TX-100 containing 5% normal goat serum and 1% BSA. The cells were immunofluorescently labelled with the H2A.Zac antibody (left) diluted 1:500 in blocking solution 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 H2A.Zac with peptides containing other histone acetylations and the unmodified H2A.Z sequence. 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. Titration of 1, 2, 5 and 10ug antibody per ChIP was analysed. IgG (2 ug/IP) was used as negative control. qPCR was performed using primers specific for the promoters of ACTB and EIF4A2 genes, used as positive control targets and for the coding region of MYT1 gene, negative control target. Image shows the recovery (the relative amount of IP'd DNA compared to input DNA). These results confirm the observation that acetylation of H2A.Z is present at active promoters.



ChIP was performed with 1 ug of the antibody against H2A.Zac on sheared chromatin from 1 million HeLaS3 cells using the “iDeal ChIP-seq” kit. IgG (2 ug/IP) was used as a negative IP control. The IP'd DNA was analysed by QPCR as described above and in 100 kb regions surrounding the EIF4A2, ACTB and GAPDH genes (figure 2D, E and F). These results clearly show an enrichment of the H2A.Z acetylation at the promoters of active genes.