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Datasheet for 600-401-X49

Histone H2A.Zac pan Antibody

Overview

Description:	Anti-Histone H2 A.Zac pan (RABBIT) Antibody - 600-401-X49
Item No.:	600-401-X49
Size:	50 µg
Applications:	ChIP, Dot Blot, ELISA, IF, WB
Reactivity:	Human
Host Species:	Rabbit

Product Details

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. Anti-Histone H2A.Zac is ideal for research in Gene Expression, Chromatin Remodeling and Epigenetics.
Synonyms:	Histone H2A.Z, H2A/z
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	IgG

Target Details

Gene Name:	H2AFZ
Reactivity:	Human

PTM Specificity:	Acetylation
Immunogen Type:	Conjugated Peptide
Immunogen:	Anti-Histone H2A.Zac Antibody was produced in rabbits by repeated immunizations with a synthetic peptide from histone H2A.Z acetylated at lysines 5, 7 and 11.
Purity/Specificity:	Anti-Histone H2A.Zac pan Antibody was purified by affinity purification. Cross reactivity with other species was not tested.
Relevant Links:	• UniProtKB - P0C0S5 • GeneID - 3015 • NCBI - NP_002097

Application Details

Tested Applications:	ChIP, Dot Blot, ELISA, IF, WB
Application Note:	Anti-Histone H2A.Zac Antibody is tested for Chromatin Immunoprecipitation, western blot, Dot Blot, ELISA, and Immunofluorescence. Specific conditions for reactivity should be optimized by the end user.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ChIP:	0.5 µg/ChIP
ELISA:	1:5,000
IF:	1:500
WB:	1:1,000

Formulation

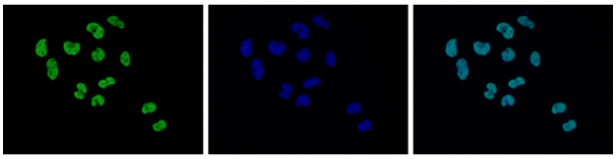
Physical State:	Liquid (sterile filtered)
Concentration:	0.7 mg/ml by UV absorbance at 280 nm
Buffer:	0.01 M Sodium Phosphate, 0.25 M Sodium Chloride, pH 7.2
Preservative:	0.05% (w/v) Sodium Azide and 0.05% ProClin 300
Stabilizer:	None

Shipping & Handling

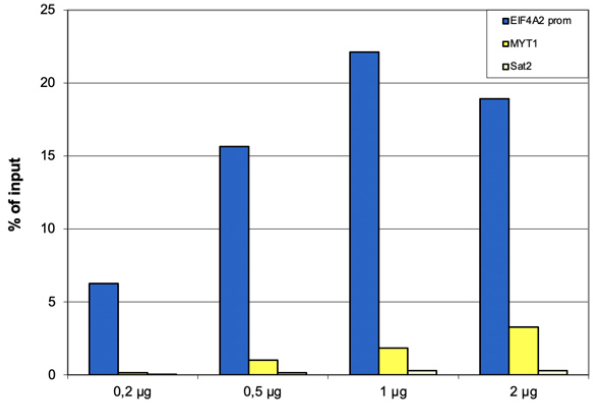
Shipping Condition:	Dry Ice

Storage Condition:	Store vial at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.
Expiration:	Expiration date is one (1) year from date of receipt.

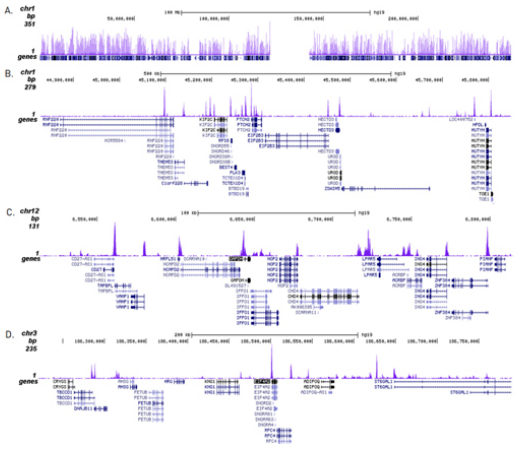
Images



Immunofluorescence Microscopy
Immunofluorescence using Anti-Histone H2A.Zac pan.
HeLa cells were stained with Anti-Histone H2A.Zac pan 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.

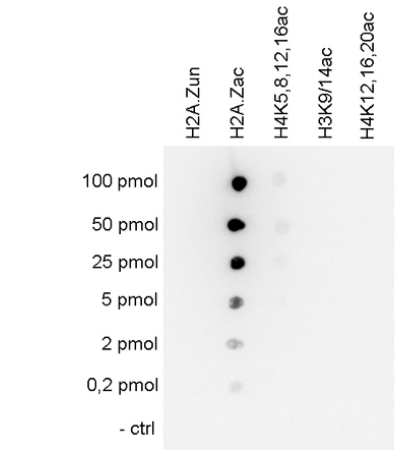


ChIP
ChIP results of Anti-Histone H2A.Zac pan.
ChIP assays were performed using HeLa cells, Anti-Histone H2A.Zac pan, and optimized primer pairs for qPCR. ChIP was performed on sheared chromatin from 100,000 K562 cells using the iDeal ChIP-seq kit. A titration of the antibody consisting of 0.2, 0.5, 1 and 2 µg per ChIP experiment was analysed. IgG (1 µg/IP) was used as negative IP control. QPCR was performed using primers specific for the promoter of the EIF4A2 gene, used as positive control target and for the coding region of the MYT1 gene, and the Sat2 satellite repeat, used as negative control targets. Figure shows the recovery (the relative amount of immunoprecipitated DNA compared to input DNA).



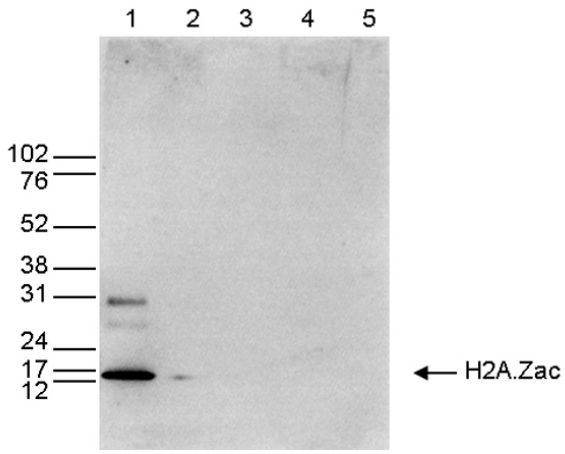
ChIP

ChIP-seq results of Anti-Histone H2A.Zac pan.
ChIP was performed with 0.5 µg of Anti-Histone H2A.Zac pan as described above. The IP'd DNA was subsequently analysed with an Illumina Genome Analyzer. Library preparation, cluster generation and sequencing were performed according to the manufacturer's instructions. The 36 bp tags were aligned to the human genome using the ELAND algorithm. Figure shows the peak distribution along the complete sequence and a 1 Mb region of human chromosome 1 (figure 2A and B) and in two regions surrounding the GAPDH and the EIF4A2 positive control gene (figure 2C and D, respectively).



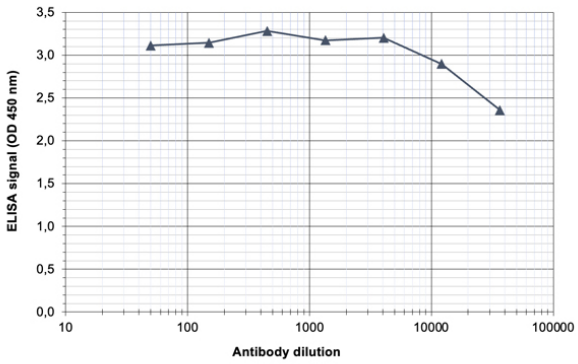
Dot Blot

Cross reactivity test using Anti-Histone H2A.Zac pan.
A Dot Blot analysis was performed to test the cross reactivity of Anti-Histone H2A.Zac pan 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 shows a high specificity of the antibody for the modification of interest.



Western Blot

Western blot analysis using Anti-Histone H2A.Zac pan.
Western blot was performed on whole cell extracts (25 µg, lane 1) from HeLa cells, and on 1 µg of recombinant histone H2A, H2B, H3 and H4 (lane 2, 3, 4 and 5, respectively) using Anti-Histone H2A.Zac pan. 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.



ELISA

Determination of the antibody titer of Anti-Histone H2AZac pan.

To determine the titer of the antibody, an ELISA was performed using a serial dilution of Anti-Histone H2AZac pan. The antigen used was a peptide containing the histone modifications of interest. By plotting the absorbance against the antibody dilution, the titer of the purified antibody was estimated to be 1:265,000.

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