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antibody and ELISA experts

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3942 B Valley Ave, Pleasanton, CA 94566
888-466-3604 | support@bosterbio.com | www.bosterbio.com

Anti-H4K5,8,12,16ac HIST1H4A Antibody

Catalog Number: CI1035

About HIST1H4A

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.

Overview

Product Name	Anti-H4K5,8,12,16ac HIST1H4A Antibody
Reactive Species	Human, Mouse
Description	Boster Bio Anti-H4K5,8,12,16ac HIST1H4A Antibody (Catalog# CI1035). Tested in ChIP, ChIP-seq, ELISA, Dot blot, IF applications. This antibody reacts with Human, Mouse.
Application	ChIP, ChIP-seq, Dot blot, ELISA, IF
Clonality	Polyclonal
Formulation	Affinity purified polyclonal antibody in PBS containing 0.05% azide and 0.05% ProClin 300.
Storage Instructions	Store at -20°C. For long-term storage, store at -80°C. Avoid multiple freeze-thaw cycles.
Host	Rabbit
Uniprot ID	P62805

Technical Details

Immunogen	This antibody is raised in rabbit against the region of histone H4 containing the acetylated lysines 5, 8, 12 and 16 (H4K5,8,12,16ac), using a KLH-conjugated synthetic peptide.
Recommended Detection Systems	Boster recommends high sensitivity ChIP-seq Kit (CK1001 & CK1002) for Chromatin Immunoprecipitation.
Form	Liquid
Concentration	0.5-1mg/ml, actual concentration vary by lot. Use suggested dilution ratio to decide dilution procedure.
Purification	Affinity purified

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Suggested Dilutions	Dilute the sample so that the expected range of concentrations fall within the detection range of this kit. If the expected range of concentration is unknown, a pilot test should be conducted to decide the optimal dilution ratio for your samples. Some PubMed article(s) citing the expression level of this target are as follows: Boster Bio's internal QC testing used: User needs to optimize the dilution ratio for this antibody.

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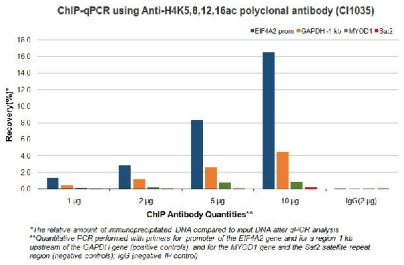
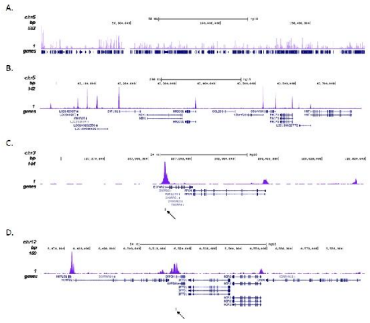
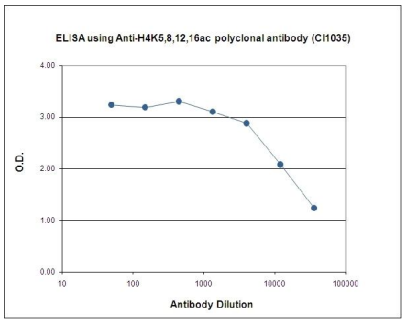
Anti-H4K5,8,12,16ac HIST1H4A Antibody (CI1035) Images



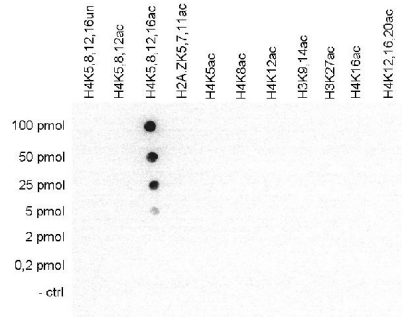
ChIP assays were performed using human HeLa cells, Anti-H4K5,8,12,16ac polyclonal antibody (Catalog # CI1035) and optimized PCR primer sets for qPCR. A titration of the antibody consisting of 1, 2, 5 and 10 ug per ChIP experiment was analysed. IgG (2 ug/IP) was used as negative IP control. QPCR was performed 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.



ChIP was performed with 2 ug of Anti-H4K5,8,12,16ac polyclonal antibody (Catalog # CI1035) on sheared chromatin from 1 million HeLa cells. The IP DNA was subsequently analysed on an Illumina Genome Analyzer. Library preparation, cluster generation and sequencing were performed according to the manufacturer instructions. The 36 bp tags were aligned to the human genome using the ELAND algorithm. Figure 2 shows the signal distribution along the complete length of chromosome 5 (figure 2A) and a zoomin to a 600 kb region (figure 2B). Figure 2C and D show the enrichment in two genomic regions on chromosome 3 and 12, respectively, containing EIF4A2 and GAPDH positive controls. The position of the amplicon used for validating the QPCR results is shown with an arrow.



To determine the titer of the antibody, an ELISA was performed using a serial dilution of Anti-H4K5,8,12,16ac polyclonal antibody (Catalog # CI1035) in antigen coated wells. The antigen used was a peptide containing the histone modification of interest. By plotting the absorbance against the antibody dilution, the titer of the antibody was estimated to be 1:21,200.



A Dot Blot analysis was performed to test the cross reactivity of Anti-H4K5,8,16ac polyclonal antibody (Catalog # CI1035) 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. This figure shows a high specificity of the antibody for the modification of interest.

Immunofluorescence images stained on NIH3T3 cells: (Left) Cells stained with anti-H4K5,8,12,16ac polyclonal antibody (Catalog # CI1035) at 1/500 dilution. (Middle) Nuclei stained

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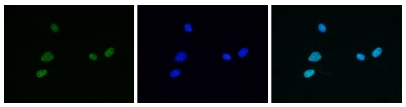

with DAPI. (Right) Merged images of two stains from the left and middle.

Anti-H4K5,8,12,16ac HIST1H4A Antibody