BOSTER
antibody and ELISA experts

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# Anti-H3K27me3 HIST1H3A Antibody

**Catalog Number:** CI1056

## About HIST1H3A

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. Trimethylation of histone H3K27 is associated with gene repression.

## Overview

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| Product Name | Anti-H3K27me3 HIST1H3A Antibody |
| Reactive Species | Drosophila, Human, Mouse, Rat, Schistosoma, Zebrafish, Arabidopsis |
| Description | Boster Bio Anti-H3K27me3 HIST1H3A Antibody (Catalog# CI1056). Tested in ChIP, ChIP-seq, ELISA, Dot blot, WB, IF applications. This antibody reacts with Drosophila, Human, Mouse, Rat, Schistosoma, Zebrafish, Arabidopsis. |
| Application | ChIP, ChIP-seq, Dot blot, ELISA, IF, WB |
| 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 | P68431 |

## Technical Details

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| Immunogen | This antibody is raised in rabbit against against histone H3, trimethylated at lysine 27 (H3K27me3), using a KLH-conjugated synthetic peptide. |
| Recommended Detection Systems | Boster recommends Enhanced Chemiluminescent Kit with anti-Rabbit IgG (EK1002) for Western blot. 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. |

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

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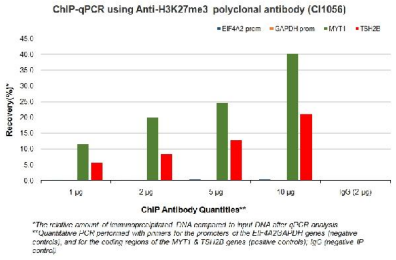
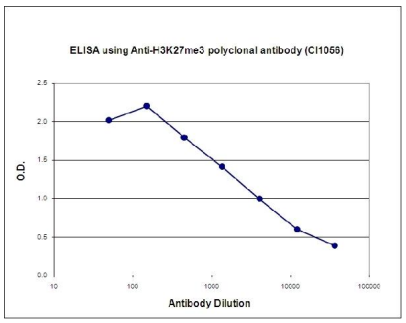
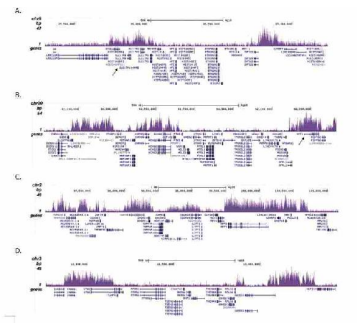
# Anti-H3K27me3 HIST1H3A Antibody (CI1056) Images



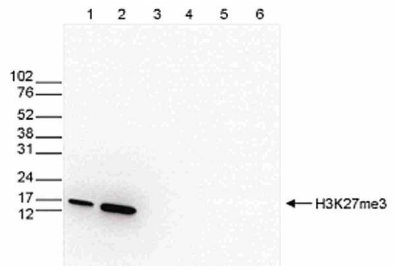

ChIP assays were performed using human HeLa cells, Anti-H3K27me3 polyclonal antibody (Catalog # CI1056) and optimized PCR primer sets for qPCR. A titration of the antibody consisting of 1, 2, 5, and 10 ug per ChIP experiment was analysed. IgG (2 ug/IP) was used as negative IP control. QPCR was performed with primers for the promoters of the active genes EIF4A2 and GAPDH as negative controls, and for the coding regions of the inactive genes MYT1 and TSH2B as positive controls.




To determine the titer of the antibody, an ELISA was performed using a serial dilution of Anti-H3K27me3 polyclonal antibody (Catalog # CI1056). 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 1:3,500.



ChIP was performed on sheared chromatin from 1 million HeLaS3 cells using 1 ug of Anti-H3K27me3 polyclonal antibody (Catalog # CI1056). The IP DNA was subsequently analysed on an Illumina HiSeq. Library preparation, cluster generation and sequencing were performed according to the manufacturer instructions. The 51 bp tags were aligned to the human genome using the BWA algorithm. Figure 2 shows the enrichment in genomic regions of chromosome 6, surrounding the TSH2B gene (indicated by an arrow; fig 2A), of chromosome 20, surrounding the MYT1 gene (fig 2B), and of chromosome 2 and 3 (figure 2C and D).




Western blot analysis of H3K27me3 expression in HeLa whole cell lysates (25 ug, lane 1), histone extracts from HeLa cells (15 ug, lane 2), recombinant histone H2A (1 ug, lane 3), recombinant histone H2B (1 ug, lane 4), recombinant histone H3 (1 ug, lane 5) and recombinant histone H4 (1 ug, lane 6). H3K27me3 was detected using Anti-H3K27me3 polyclonal antibody (Catalog # CI1056) at 1/500 dilution.

A Dot Blot analysis was performed to test the cross reactivity of Anti-H3K27me3 polyclonal antibody (Catalog # CI1056) with peptides containing other modifications of histone H3 and H4 and the unmodified H3K27 sequence. One hundred to 0.2 pmol of the peptide containing the respective histone modification were spotted on a membrane. The antibody was used at a dilution of 1:5,000. This figure shows a high specificity of the antibody for the modification of interest. Please note that that antibody also

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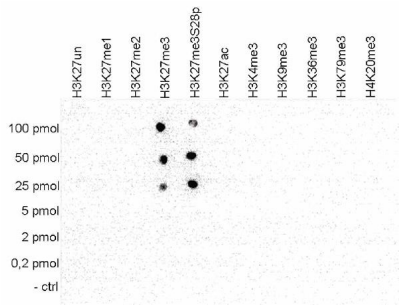
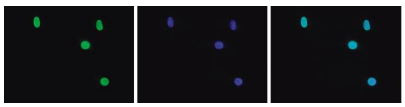



recognizes the modification if S28 is phosphorylated.



Immunofluorescence images stained on mouse NIH3T3 cells: (Left) Cells stained with anti-H3K27me3 polyclonal antibody (Catalog # CI1056) at 1/200 dilution. (Middle) Nuclei stained with DAPI. (Right) Merged images of two stains from the left and middle.



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