BioVision

08/14

For research use only

H3K27me3S28p Antibody

ALTERNATE NAMES: Histone H3

CATALOG #: 6810-50

AMOUNT: 50 µl

HOST/ISOTYPE: Rabbit

IMMUNOGEN: KLH-conjugated synthetic peptide of Histone H3 containing trimethylated lysine 27 and the phosphorylated serine 28.

FORM: Liquid

FORMULATION: In PBS with 0.05% (W/V) sodium azide.

PURIFICATION: Whole antiserum from rabbit

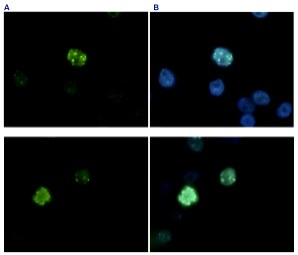
SPECIES REACTIVITY: Human.

STORAGE CONDITIONS: Store at -20°C; for long storage, store at -80°C. Avoid multiple freeze-thaw cycles.

DESCRIPTION: 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. 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. Histone methylation and demethylation is dynamically regulated by respectively histone methyl transferases and histone demethylases. Phosphorylation of H3 on serine 28 is increased during mitosis.

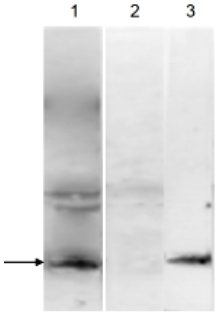
APPLICATION: IF: 1:200, WB: 1:250 – 1:500, ELISA: 1:100 – 1:500, Dot Blot: 1:20,000, ChIP: 1 µl/ChIP, IP: 5 µl/IP.

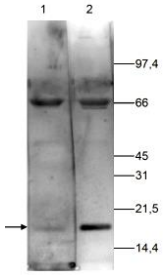
Note: This information is only intended as a guide. The optimal dilutions must be determined by the user.



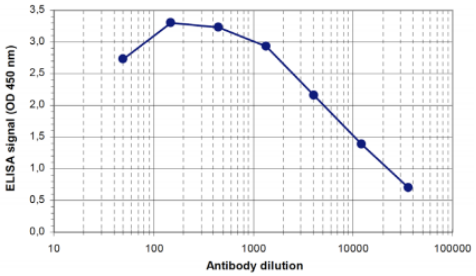
HeLa asynchronous cells were stained with the antibody against and DAPI. Fig A: cells were labelled with antibody. Fig B: the nuclei were stained with DAPI. Phosphorylation of H3 on serine 28 is increased during late G2 phase and reaches a maximum in metaphase cells. This may explain the different staining intensities of different cells.

HeLa cells were treated with colcemid to block the cell cycle in metaphase. IP was performed with 5 µl of the antibody. The IPed proteins were analysed by WB with the antibody diluted 1:500 Lane 1 shows the result of the IP; a negative IP control (no antibody added) and a positive control (sheared chromatin from 10,000 cells) are shown in lane 2 and 3, respectively.

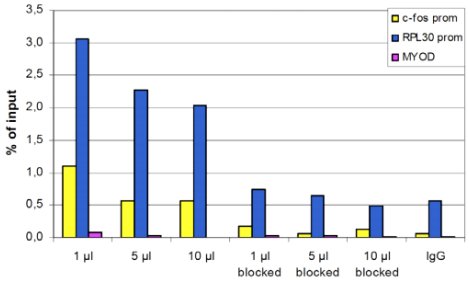




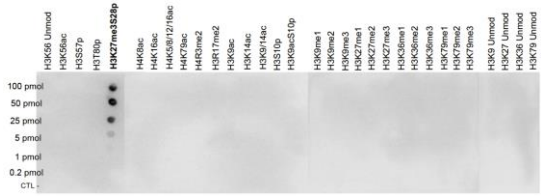
HeLa cells extracts (15 µg) were analysed by WB blot using the antibody.



To determine the titer, an ELISA was performed using a serial dilution of the antibody. 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 1:8300.



ChIP assays were performed using HeLa cells and the antibody and optimized PCR primer sets for qPCR. A titration of the antibody consisting of 2, 5, 10 and 15 µl per ChIP experiment was analysed. IgG (5 µg/IP) was used as negative control. The Fig shows the recovery, expressed as a % of input (the relative amount of IP DNA compared to input DNA after qPCR analysis).



A Dot Blot analysis was performed to test the cross reactivity of the antibody with peptides containing other modifications of histone H4 and H3 and unmodified H3 sequences. 100 to 0.2 pmol of the peptide containing the respective histone modification were spotted on a membrane. The Fig shows a high specificity of the antibody for the modification of interest.

RELATED PRODUCTS:

- H3R17me2 Antibody **(Cat # 6803-50)**
- H3K9me2 Antibody **(Cat # 6804-50)**
- H3K36me2 Antibody **(Cat # 6805-50)**
- H3 Pan Antibody **(Cat # 6806-50)**
- H4K8ac Antibody **(Cat # 6807-50)**

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BioVision Incorporated
155 S. Milpitas Boulevard, Milpitas, CA 95035 USA

Tel: 408-493-1800 | Fax: 408-493-1801
www.biovision.com | tech@biovision.com