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Product datasheet for TA347165

H3FA (HIST1H3A) Rabbit Polyclonal Antibody

Product data:

Product Type:	Primary Antibodies
Applications:	Dot, ELISA, IF, WB
Recommended Dilution:	ChIP (1:5,000); ELISA (1:1,000); Dot blotting (1:10,000); Western blotting (1:750); Immunofluorescence (1:200)
Reactivity:	Human, Mouse
Host:	Rabbit
Isotype:	IgG
Clonality:	Polyclonal
Immunogen:	The immunogen for anti-H3K9me3 antibody: histone H3 containing the trimethylated lysine 9 (H3K9me3), using a KLH-conjugated synthetic peptide.
Concentration:	lot specific
Purification:	Whole antiserum from rabbit containing 0.05% azide.
Conjugation:	Unconjugated
Storage:	Store at -20°C as received.
Stability:	Stable for 12 months from date of receipt.
Gene Name:	histone cluster 1, H3a
Database Link:	NP_003520 Entrez Gene 360198 MouseEntrez Gene 8350 Human P68431

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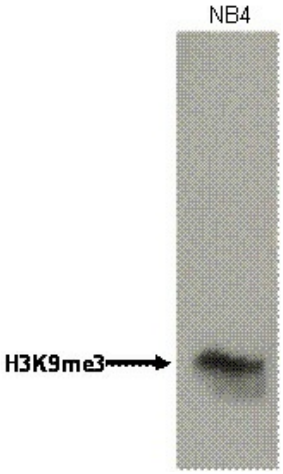
Background: Histones are the main constituents of the protein part of chromosomes of eukaryotic cells. They are rich in the amino acids arginine and lysine and have been greatly conserved during evolution. Histones pack the DNA into tight masses of chromatin. Two core histones of each class H2A, H2B, 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. Trimethylation of histone H3K9 is associated with heterochromatin formation and gene silencing.

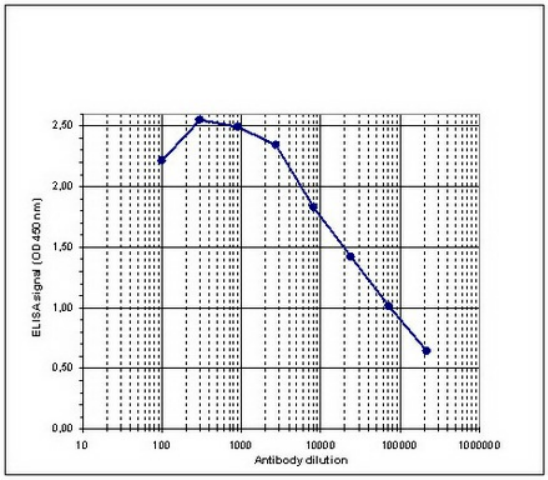
Synonyms: A; H3; H3FA

Protein Pathways: Systemic lupus erythematosus

Product images:

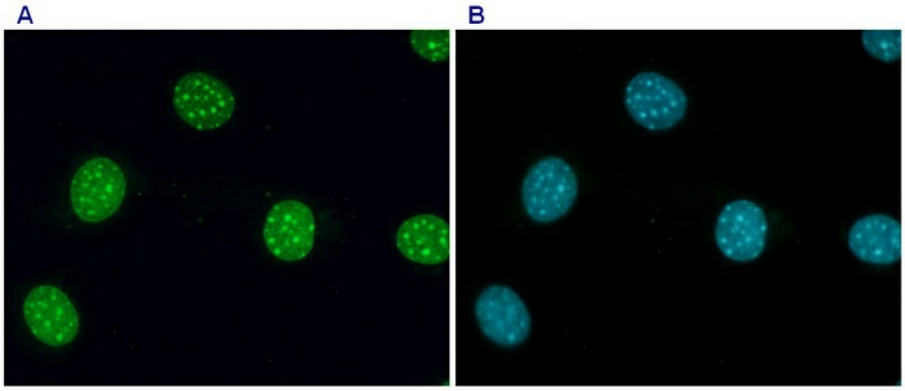


WB using the antibody against H3K9me3 diluted 1:750 in TBS-Tween containing 5% skimmed milk. The position of the protein of interest of interest is indicated on the left.

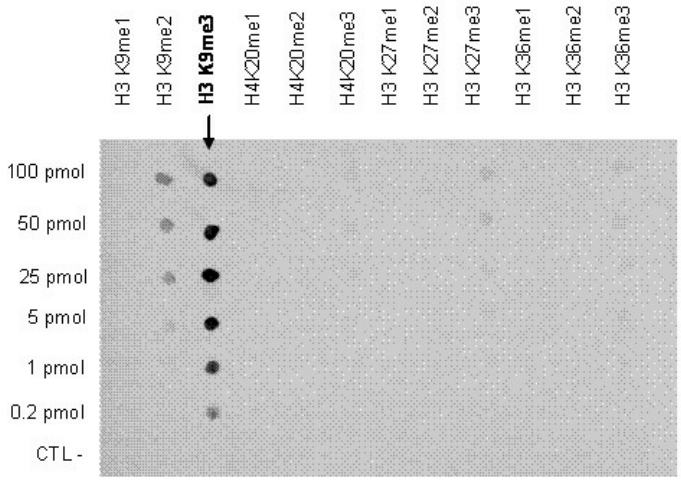


Determination of the antibody titer To determine the titer of the antibody, an ELISA was performed using a serial dilution of the antibody against H3K9me3. The antigen used was a peptide containing the histone modification of interest. By plotting the absorbance against the antibody dilution (Figure 2), the titer of the antibody was estimated to be 1:35,000.

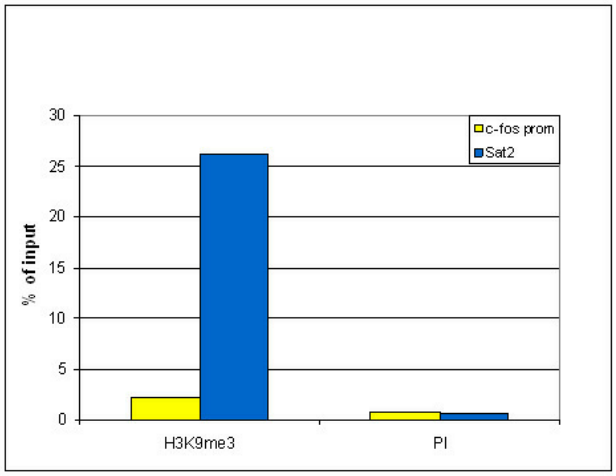
Figure 5



NIH3T3 cells were stained with the ab and DAPI. Cells were formaldehyde fixed, permeabilized with TritonX100 and blocked with PBS containing 2.5% BSA. A: cells were labelled with anti-H3K9me3 (1:200 and incubated for 1 hr at RT) followed by goat anti-rabbit conjugated to FITC. B: staining of the nuclei with DAPI. Both antibody and DAPI staining are restricted to the nucleus. The distribution pattern of H3K9me3 is linked to the transcriptionally inactive, condensed pericentric heterochromatin.



Dot Blot performed to test the cross reactivity of the ab with peptides containing other histone modifications of histone H3 and H4. Other histone modifications include mono- and dimethylation of the same lysine and mono-, di- and trimethylation of lysine 27 and 36 of H3, and of lysine 20 of H4. One hundred to 0.2 pmol of peptide containing the respective histone modification were spotted on a membrane. The ab was used at 1:10,000. Image shows a high specificity of the antibody.



ChIP assays using undifferentiated human teratocarcinoma cells (NCCIT). Sheared chromatin from 10,000 cells was used per ChIP and the ab at 1:5000. The pre-immune serum (PI, diluted 1:5000) was negative control. qPCR primer were sets for the satellite repeat Sat2 as a positive control and for the promoter of the house keeping gene c-fos as a negative control. Image shows the recovery, expressed as a % of input (the relative amount of IP'd DNA compared to input DNA after qPCR). Results are in accordance that H3K9me3 is preferably present at heterochromatin.