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Product datasheet for TA347189

H3FA (HIST1H3A) Rabbit Polyclonal Antibody

Product data:

Product Type:	Primary Antibodies
Applications:	Dot, ELISA, IF, WB
Recommended Dilution:	ChIP (2 µg/ChIP); ELISA (1:1,000); Dot blotting (1:20,000) ; Western blotting (1:1,000) ; Immunofluorescence (1:500)
Reactivity:	Human, Mouse
Host:	Rabbit
Isotype:	IgG
Clonality:	Polyclonal
Immunogen:	The immunogen for anti-H3K9me2 antibody: the region of histone H3 containing the dimethylated lysine 9 (H3K9me2), using a KLH-conjugated synthetic peptide.
Concentration:	lot specific
Purification:	Affinity purified polyclonal antibody in PBS containing 0.05% azide and 0.05% ProClin 300.
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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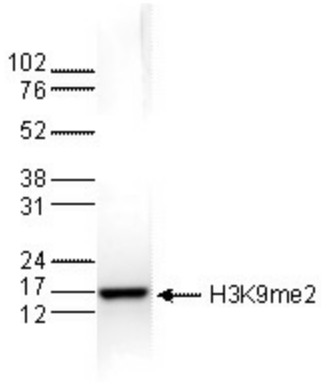
Background: Histones are the main constituents of the protein part of chromosomes of eukaryotic cells. They are rich in the amino acids arginine and lysine and have been greatly conserved during evolution. Histones pack the DNA into tight masses of chromatin. Two core histones of each class H2A, H2B, 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. Dimethylation of histone H3K9 is more present in silent genes.

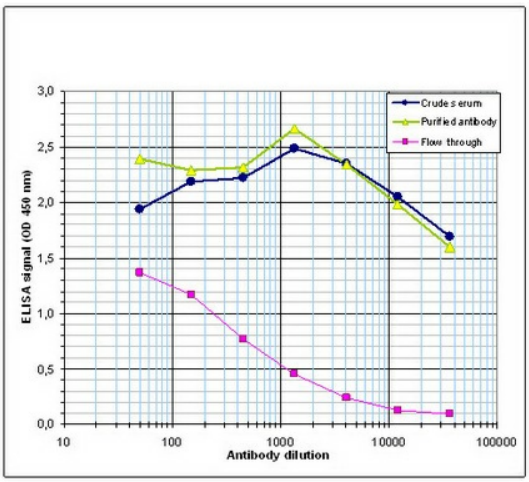
Synonyms: A; H3; H3FA

Protein Pathways: Systemic lupus erythematosus

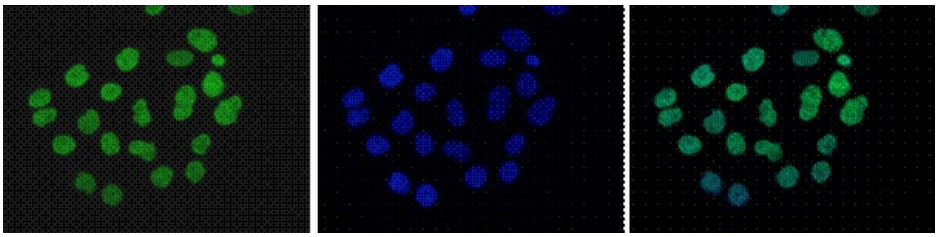
Product images:



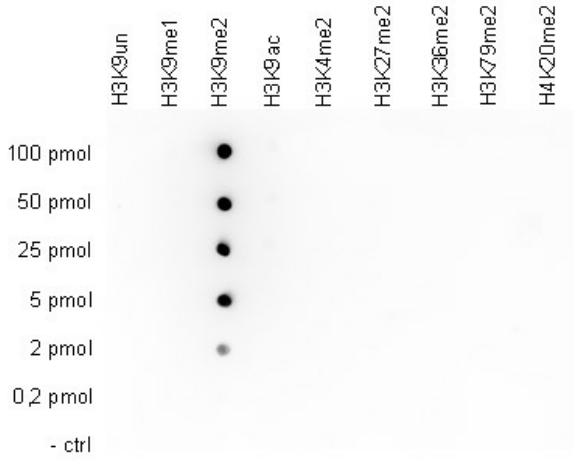
WB using the antibody against H3K9me2 diluted 1:1,000 in TBS-Tween containing 5% skimmed milk. The position of the protein of interest is indicated on the right; the marker (in kDa) is shown on the left.



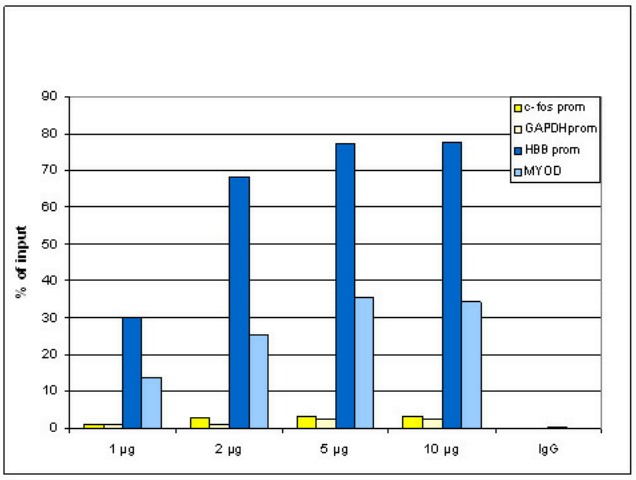
Determination of the antibody titer To determine the titer of the antibody, an ELISA was performed using a serial dilution of the antibody against H3K9me2, crude serum and Flow through. The antigen used was a peptide containing the histone modification of interest. By plotting the absorbance against the antibody dilution (Figure 2), the titer of the antibody was estimated to be 1:103,000.



INIH3T3 cells were stained with the ab against H3K9me2 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 labeled with the H3K9me2 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.



A Dot Blot analysis was performed to test the cross reactivity of the antibody against H3K9me2 with peptides containing other modifications of histone H3 and the unmodified H3K9 sequence. One hundred to 0.2 pmol of peptide containing the respective histone modification were spotted on a membrane. The antibody was used at a dilution of 1:20,000. Image shows a high specificity of the antibody for the modification of interest.



ChIP assays using HeLa cells (sheared chromatin from 1 million cells). Titration of 1, 2, 5, and 10ug antibody per ChIP was analysed. IgG (2 ug/IP) was used as negative control. qPCR primers were specific for promoter of inactive HBB gene and the coding regions of inactive MYOD gene as positive controls, and for the promoters of active genes c-fos and GAPDH as negative controls. Image shows the recovery, expressed as a % of input (the relative amount of IP'd DNA compared to input DNA after PCR).