OriGene Technologies, Inc.
9620 Medical Center Drive, Ste 200
Rockville, MD 20850, US
Phone: +1-888-267-4436
https://www.origene.com
techsupport@origene.com
EU: info-de@origene.com
CN: techsupport@origene.cn

Product datasheet for TA347199

H3FA (HIST1H3A) Rabbit Polyclonal Antibody

Product data:

Product Type:	Primary Antibodies
Applications:	Dot, ELISA, IF, WB
Recommended Dilution:	ChIP/ChIP-seq (1ug/IP); ELISA (1:1,000); Dot blotting (1:20,000); Western blotting (1:500); Immunofluorescence (1:500)
Reactivity:	Human, Mouse, Broad
Host:	Rabbit
Isotype:	IgG
Clonality:	Polyclonal
Immunogen:	The immunogen for anti-H3R17me2(asym)K18ac antibody: the region of histone H3 containing the asymmetrically dimethylated R17 and the acetylated lysine 18 (H3R17me2(asym)K18ac), 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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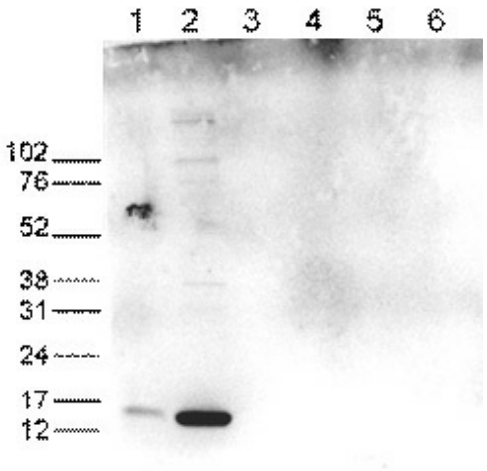
Background: Histones are the main constituents of the protein part of chromosomes of eukaryotic cells. They are rich in the amino acids arginine and lysine and have been greatly conserved during evolution. Histones pack the DNA into tight masses of chromatin. Two core histones of each class H2A, H2B, 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.

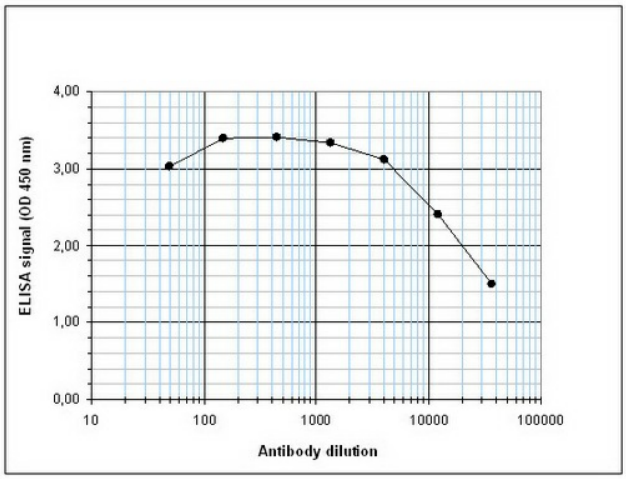
Synonyms: A; H3; H3FA

Protein Pathways: Systemic lupus erythematosus

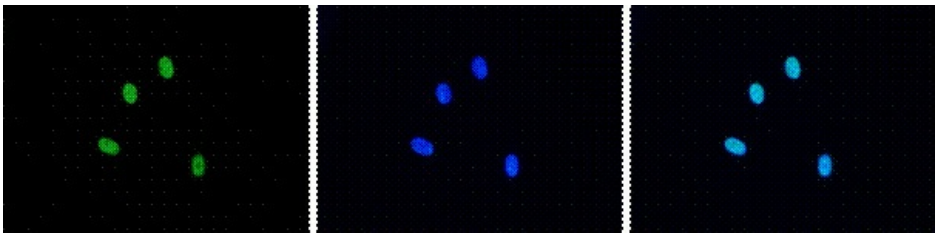
Product images:



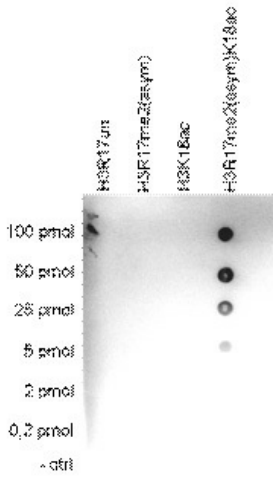
WB was performed on whole cell (25 ug, lane 1) and histone extracts (15 ug, lane 2) from HeLa cells, and on 1 ug of recombinant histone H2A, H2B, H3 and H4 (lane 3, 4, 5 and 6, respectively) using the antibody against H3R17me2 (asym) K18ac. The antibody was diluted 1:1,000 in TBS-Tween containing 5% skimmed milk. The marker (in kDa) is shown on the left.



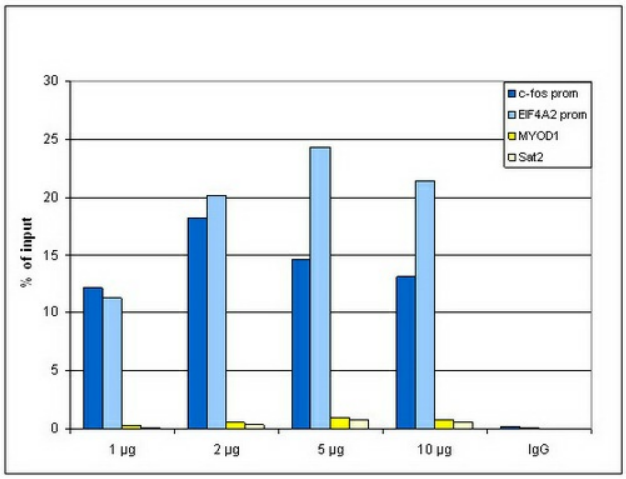
Determination of the antibody titer To determine the titer of the antibody, an ELISA was performed using a serial dilution of the antibody against H3R17me2 (asym) K18ac. The antigen used was a peptide containing the histone modification of interest. By plotting the absorbance against the antibody dilution (Figure 3), the titer of the antibody was estimated to be 1:28, 800.



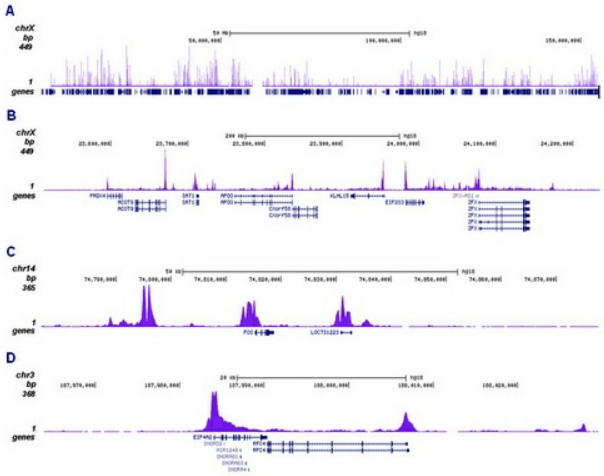
INIH3T3 cells were stained with the ab against H3R17me2 (asym)K18ac and with DAPI. Cells were fixed with 4% formaldehyde for 10' and blocked with PBS/TX-100 containing 5% normal goat serum and 1% BSA. The cells were immunofluorescently labeled with the H3R17me2 (asym)K18ac antibody (left) diluted 1:500 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 with peptides containing other histone modifications and the unmodified H3R17K18. 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. Figure 4 shows a high specificity of the antibody for the modification of interest.



ChIP assays using HeLa cells: ChIP-seq" kit, using sheared chromatin from 1,000,000 cells. A titration of 1, 2, 5 and 10 ug ab was used. IgG (2 ug/IP) was negative IP control. qPCR was performed with primers for the promoters of the active EIF4A2 and c-fos genesas positive controls and for the inactive MYOD1 gene and the Sat2 satellite repeatas negative controls. Image shows the recovery, expressed as a % of input (the relative amount of IP'd DNA compared to input DNA after qPCR analysis).



ChIP was performed as described above using 1 ug of the ab against H3R17me2 (asym) K18ac. The IP'd DNA was subsequently analysed on an Illumina Genome Analyzer. The 36 bp tags were aligned to the human genome using the ELAND algorithm. Image shows the peak distribution along the complete human X-chromosome and a zoomin to a 500 kb region (figure 2A and B), and in two regions on chromosome 14 and 3 surrounding the c-fos and EIF4A2 positive control genes (figure 2C and D, respectively).