

## Product datasheet for **TA347174**

### H3FA (HIST1H3A) Rabbit Polyclonal Antibody

#### Product data:

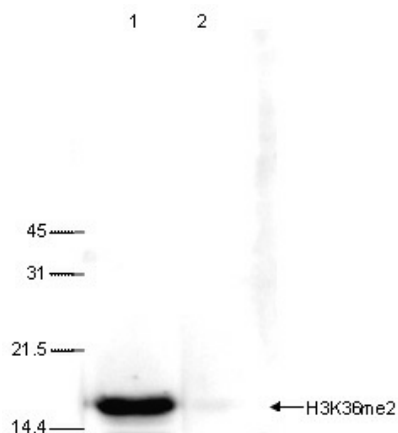
Product Type:	Primary Antibodies
Applications:	Dot, ELISA, IF, WB
Recommended Dilution:	ChIP/ChIP-seq (5 µl/IP); ELISA (1:1,000); Dot blotting (1:100,000); Western blotting (1:1,000); Immunofluorescence (1:500)
Reactivity:	Human
Host:	Rabbit
Isotype:	IgG
Clonality:	Polyclonal
Immunogen:	The immunogen for anti-H3K36me2 antibody: histone H3 containing the dimethylated lysine 36 (H3K36me2), 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:	<a href="#">NP_003520</a> <a href="#">Entrez Gene 8350 Human</a> <a href="#">P68431</a>
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.


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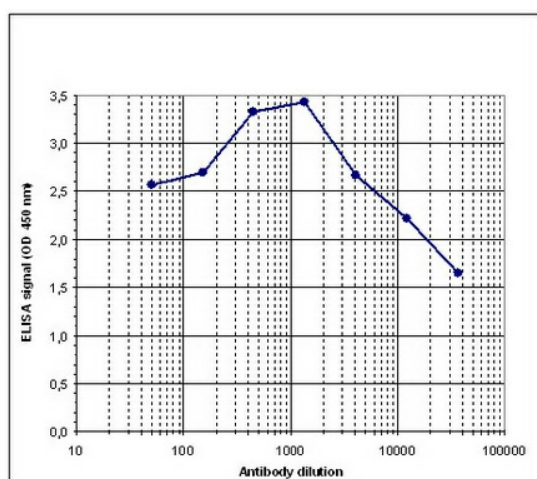
**Synonyms:** A; H3; H3FA

**Protein Pathways:** Systemic lupus erythematosus

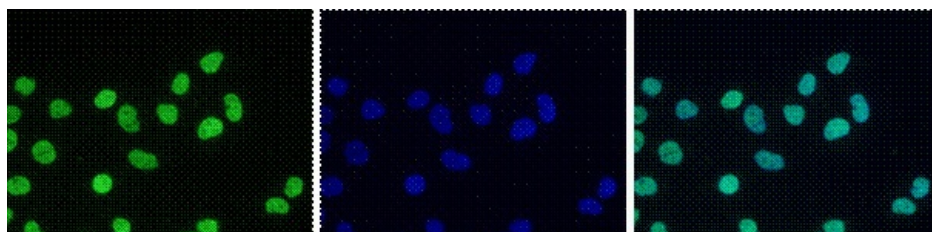
## Product images:



WB using the antibody against H3K36me2 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. The result of the Western analysis with the antibody is shown in lane 1; lane 2 shows the same analysis after incubation of the antibody with 5 nmol blocking peptide for 1 hour at room temperature.



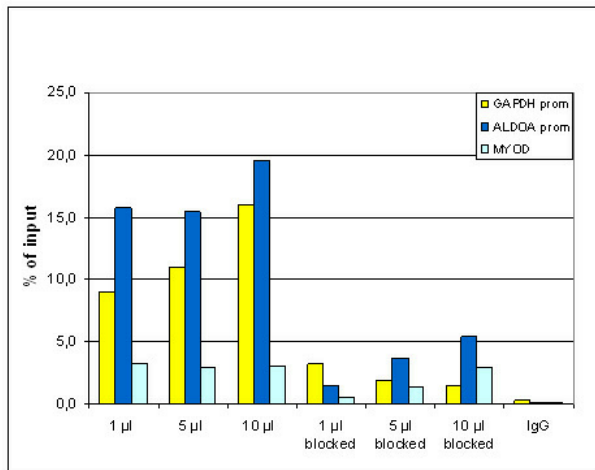
**Determination of the titer** To determine the titer, an ELISA was performed using a serial dilution of the antibody against H3K36me2. The antigen used was a peptide containing the histone modification of interest. By plotting the absorbance against the antibody dilution (Figure 3), the titer of the antibody was estimated to be 1:31,000.



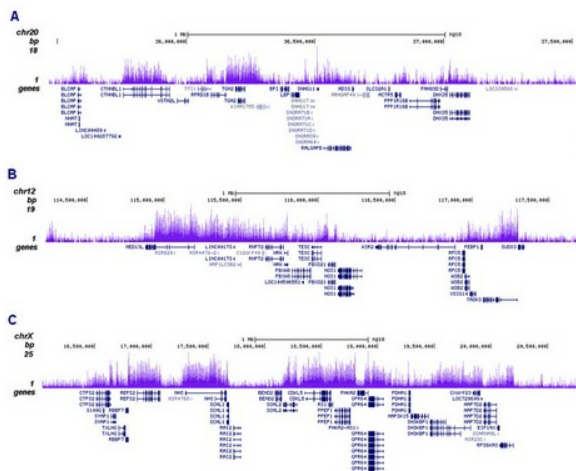
HeLa cells were stained with the antibody against H3K36me2 and with DAPI. Cells were fixed with 4% formaldehyde for 10' and blocked with PBS/TX-100 containing 5% normal goat serum and 1% BSA. The cells were immunofluorescently labelled with the H3K36me2 antibody (left) diluted 1:500 in blocking solution 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 H3K36me2 with peptides containing other modifications and unmodified sequences of histone H3. 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 1:100,000. Figure 4 shows a high specificity of the antibody for the modification of interest.



ChIP assays using HeLa cells (sheared chromatin from 10,000 cells). Titration of 1, 5, and 10 µl antibody per ChIP was analysed. Additionally, the same titration was analyzed after incubation with 5 nM blocking peptide for 1 hr at RT. IgG (5 µg/IP) was used as negative control. qPCR primers were for the promoter of active genes GAPDH and ALDOA and for the coding region of MYOD. Image shows the recovery, expressed as a % of input (the relative amount of IP'd DNA compared to input DNA after qPCR).



ChIP was performed with 0.5 µl of the ab against H3K36me2 on sheared chromatin from 1 million HeLa cells using the "iDeal ChIP-seq" kit. 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 signal distribution along 3 genomic regions of chromosome 20, 12 and X, respectively.