

PRODUCT NAME		
H3K36me1 polyclonal antibody		
Cat. No. <b>C15410089</b> (pAb-089-050)	Type: Polyclonal ChIP-grade	Size: 50 µg/ 54 µl
Lot #: A2367-001P	Source: Rabbit	Concentration: 0.94 µg/µl

**Product description:** Polyclonal antibody raised in rabbit against histone H3 containing the monomethylated lysine 36 (H3K36me1), using a KLH-conjugated synthetic peptide.

**Specificity:** Human: positive  
Other species: not tested

Applications	Suggested dilution	References
ChIP*	5 µg/ChIP	Fig 1
ELISA	1:100	Fig 2
Dot blotting	1:20,000	Fig 3
Western blotting	1:1,000	Fig 4
IF	1:500	Fig 5

\*Please note that of the optimal antibody amount per IP should be determined by the end-user. We recommend testing 1-5 µg per IP.

**Purity:** Affinity purified polyclonal antibody in PBS containing 0.05% azide and 0.05% ProClin 300.

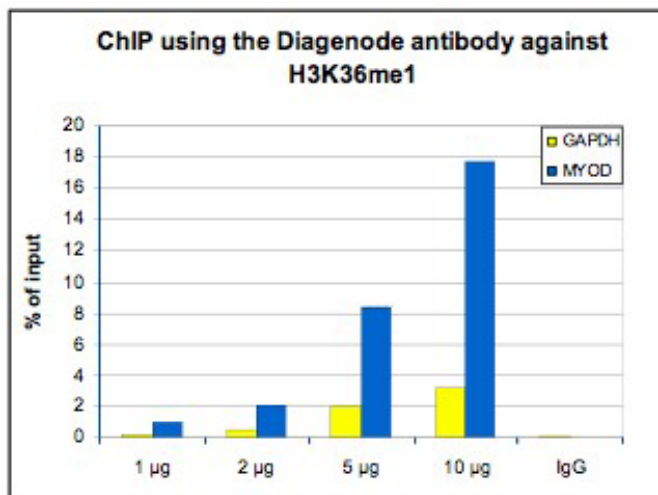
**Storage:** Store at -20°C; for long storage, store at -80°C. Avoid multiple freeze-thaw cycles.

**Precautions:** This product is for research use only. Not for use in diagnostic or therapeutic procedures.

**Last data sheet update:** June 10, 2011

**Target description**

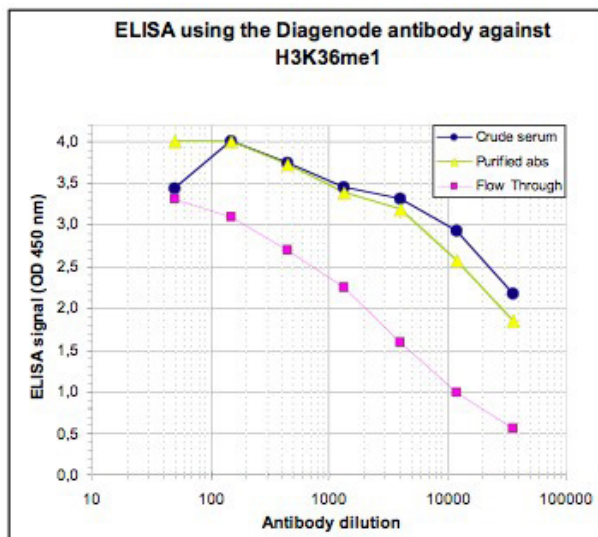
Histones are the main constituents of the protein part of chromosomes of eukaryotic cells. They are rich in the amino acids arginine and lysine and have been greatly conserved during evolution. Histones pack the DNA into tight masses of chromatin. Two core histones of each class H2A, H2B, H3 and H4 assemble 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.



**Figure 1**

**ChIP results obtained with the Diagenode antibody directed against H3K36me1**

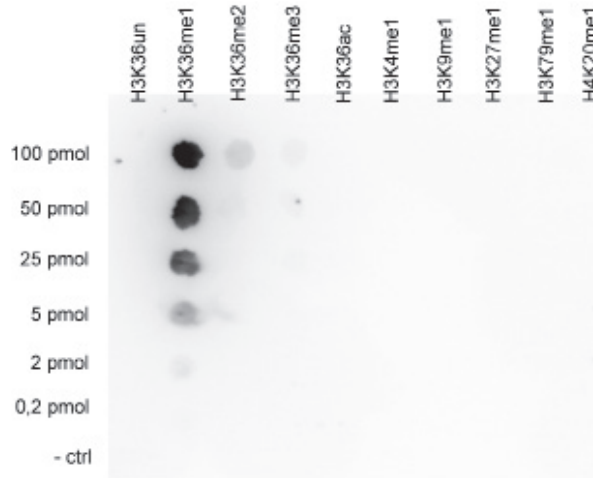
ChIP assays were performed using human U2OS cells, the Diagenode antibody against H3K36me1 (cat. No. pAb-089-050) and optimized PCR primer pairs for qPCR. ChIP was performed with the “OneDay ChIP” kit (cat. No. kch-oneDIP-060), using sheared chromatin from 1.5 million cells and stringent washing conditions. A titration consisting of 1, 2, 5 and 10 µg of antibody per ChIP experiment was analyzed. IgG (1 µg/IP) was used as a negative IP control. Quantitative PCR was performed with primers for the promoter of the constitutively expressed GAPDH gene and for the inactive gene MYOD. Figure 1 shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).



**Figure 2**

**Determination of the antibody titer**

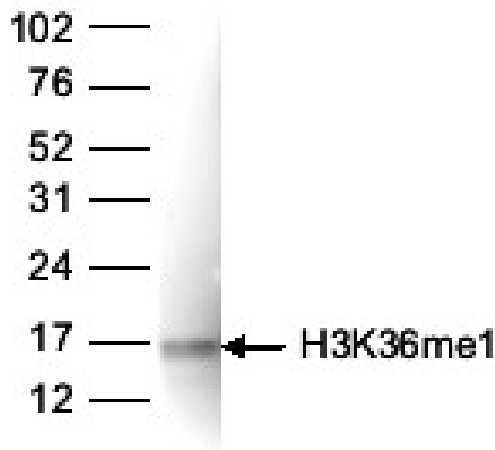
To determine the titer of the antibody, an ELISA was performed using a serial dilution of the Diagenode antibody directed against H3K36me1 (cat. No. pAb-089-050), crude serum and Flow Through. The antigen used was a peptide containing the histone modification of interest. By plotting the absorbance against the antibody dilution (Figure 2), the titer of the antibody was estimated to be 1:46,000.



**Figure 3**

**Cross reactivity tests using the Diagenode antibody directed against H3K36me1**

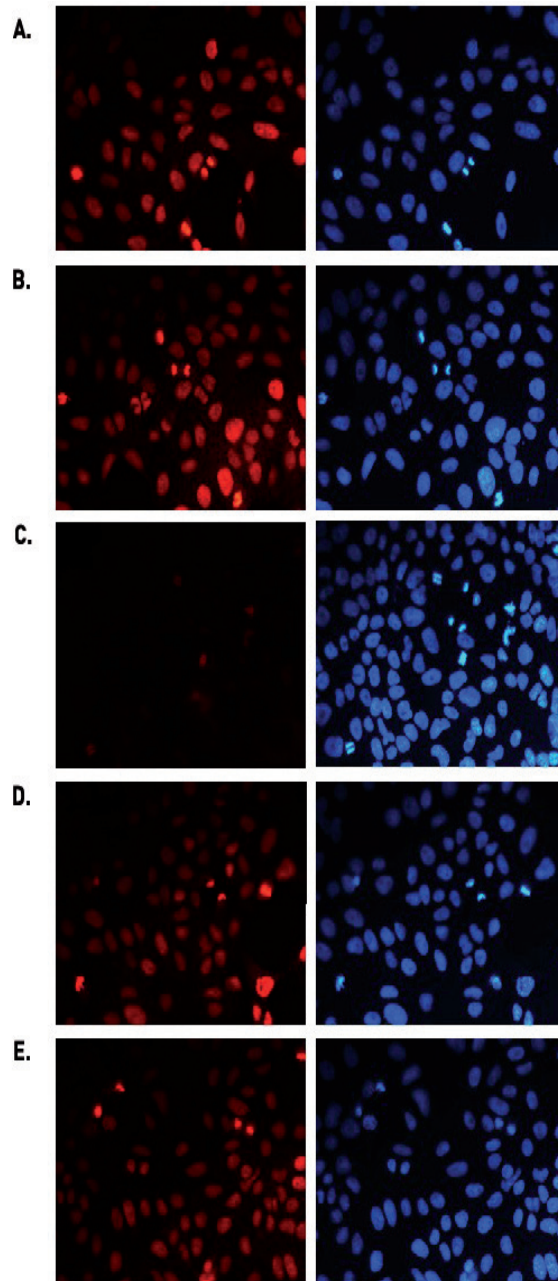
A Dot Blot analysis was performed to test the cross reactivity of the Diagenode antibody against H3K36me1 (cat. No. pAb-089-050) with peptides containing other modifications of histone H3 or the unmodified H3K36. One hundred to 0.2 pmol of the peptide containing the respective histone modification were spotted on a membrane. The antibody was used at a dilution of 1:20,000. Figure 3 shows a high specificity of the antibody for the modification of interest.



**Figure 4**

**Western blot analysis using the Diagenode antibody directed against H3K36me1**

Histone extracts (15 µg) from HeLa cells were analysed by Western blot using the Diagenode antibody against H3K36me1 (cat. No. pAb-089-050) 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.



**Figure 5**

**Immunofluorescence using the Diagenode antibody directed against H3K36me1**

Human osteosarcoma [U2OS] cells were stained with the Diagenode antibody against H3K36me1 (cat. No. pAb-089-050) and with DAPI. Cells were fixed with 4% formaldehyde for 20' and blocked with PBS/TX-100 containing 5% normal goat serum. Figure 5A: cells were immunofluorescently labeled with the H3K36me1 antibody (left) diluted 1:500 in blocking solution followed by an anti-rabbit antibody conjugated to Alexa568 or with DAPI (right), which specifically labels DNA. Figure 5B, C, D and E: staining of the cells with the H3K36me1 antibody after incubation of the antibody with 2 ng/ $\mu$ l blocking peptide containing the unmodified and the mono-, di- and trimethylated H3K36, respectively.