

PRODUCT NAME H3K36me3 polyclonal antibody		
Cat. No. pAb-058-050	Type: Polyclonal ChIP grade	Size: 50 µg/ 50 µl
Lot #: A241-0011	Source: Rabbit	Concentration: 1 µg/µl

Description: Polyclonal antibody raised in rabbit against histone H3, trimethylated at lysine 36 (H3K36me3), using a KLH-conjugated synthetic peptide.

Specificity: Human: positive
Other species: not tested

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

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.

References citing this antibody:

(1) Marks H, Chow JC, Denissov S, François KJ, Brockdorff N, Heard E and Stunnenberg HG (2009) High-resolution analysis of epigenetic changes associated with X inactivation. *Genome Res* 19:1361-73.

Last data sheet update: March 1, 2010

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. Trimethylation of H3K36 is associated with actively transcribed regions.

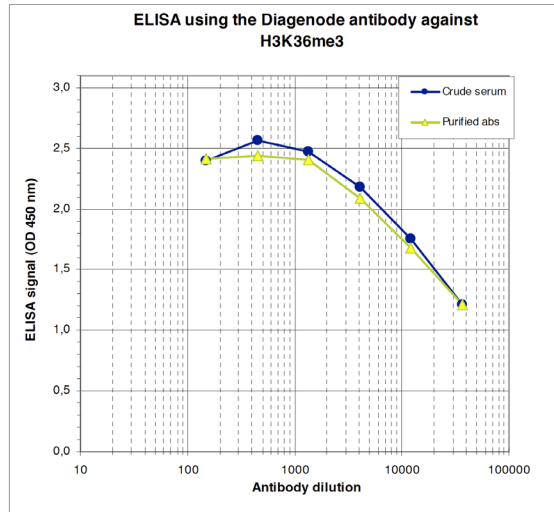


Figure 1
Determination of the antibody titer

To determine the titer of the antibody, an ELISA was performed using a serial dilution of the Diagenode antibody directed against H3K36me3 (Cat. No. pAb-058-050) and the crude serum. The antigen used was a peptide containing the histone modification of interest. By plotting the absorbance against the antibody dilution (Figure 1), the titer of the purified antibody was estimated to be 1:39,250.

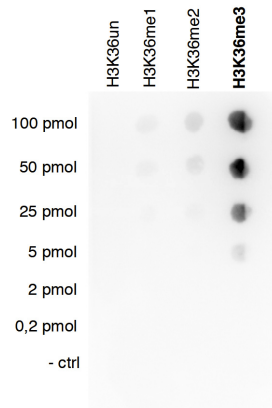


Figure 2
Cross reactivity tests using the Diagenode antibody directed against H3K36me3

A Dot Blot analysis was performed to test the cross reactivity of the Diagenode antibody against H3K36me3 (Cat. No. pAb-058-050) with peptides containing other H3K36 methylations and the unmodified 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 1:100,000. Figure 2 shows a high specificity of the crude serum for the modification of interest.

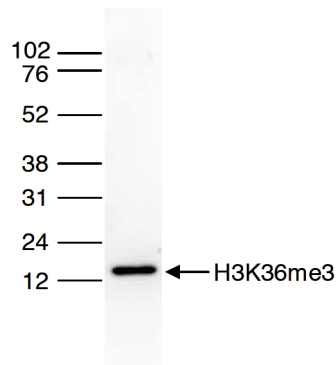


Figure 3

Western blot analysis using the Diagenode antibody against H3K36me3

Histone extracts (15 µg) from HeLa cells were analysed by Western blot using the Diagenode purified antibody directed against H3K36me3 (Cat. No. pAb-058-050) 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.

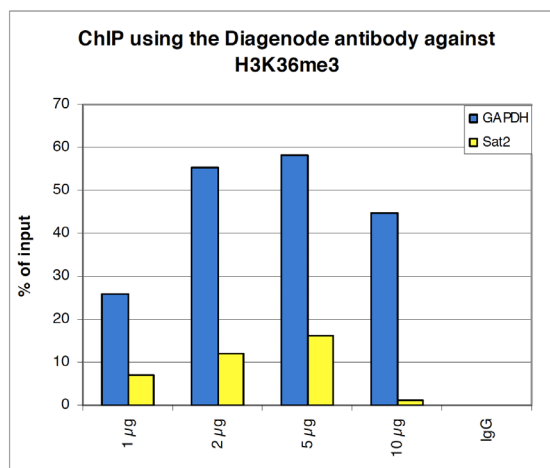


Figure 4

ChIP results obtained with the Diagenode antibody directed against H3K36me3

ChIP assays were performed using human osteosarcoma (U2OS) cells, the Diagenode antibody against H3K36me3 (Cat. No. pAb-058-050) and optimized PCR primer sets for qPCR. Chromatin was sheared with the Diagenode “Shearing ChIP” kit (Cat. No. kch-redmod-100). ChIP was performed with the “OneDay ChIP” kit (Cat. No. kch-oneDIP-060), using sheared chromatin from 1.5 million cells. A titration of the antibody consisting of 1, 2, 5 and 10 µg per ChIP experiment was analysed. IgG (1 µg/IP) was used as a negative IP control. Figure 4 shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis), of the housekeeping gene GAPDH and of the satellite repeat Sat2. These results are in accordance with the observation that H3K36me3 is enriched at active genes.