

H3K36me3 Antibody - ChIP-seq Grade

Cat. No. C15410058

Type: Polyclonal	Specificity: Human, mouse, zebrafish, Drosophila: positive. Other species: not tested.
Size: 50 µg	Isotype: NA
Concentration: 0.9 µg/µl	Host: Rabbit
Lot No.: A8889-001P	Purity: Affinity purified
Storage buffer: PBS containing 0.05% azide and 0.05% ProClin 300.	Storage conditions: 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.	

Description

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

Applications

Applications	Suggested dilution	References
ChIP/ChIP-seq *	1-2 µg/ChIP	Fig 1, 2
ELISA	1:500	Fig 3
Dot Blotting	1:20,000	Fig 4
Western Blotting	1:1,000	Fig 5

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

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.

Validation Data

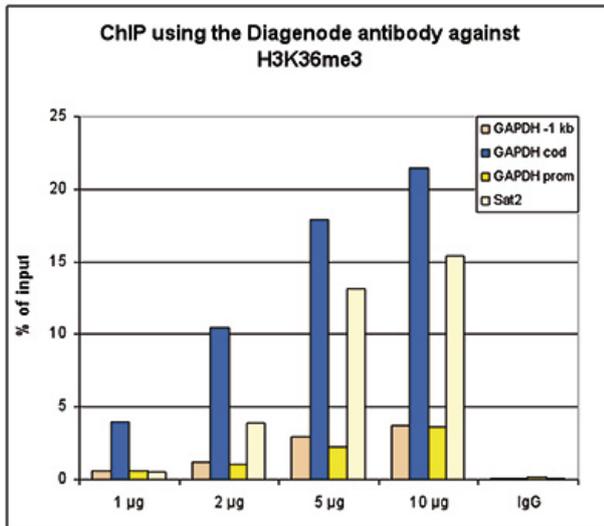


Figure 1. ChIP results obtained with the Diagenode antibody directed against H3K36me3 ChIP assays were performed using HeLa cells, the Diagenode antibody against H3K36me3 (cat. No. C15410058) and optimized PCR primer sets for qPCR. ChIP was performed with the “Auto Histone ChIP-seq” kit (cat. No. C01010022), using sheared chromatin from 1 million cells on the SX-8G IP-Star automated system. A titration consisting of 1, 2, 5 and 10 µg of antibody per ChIP experiment was analyzed. IgG (2 µg/IP) was used as a negative IP control. QPCR was performed with primers for the promoter and coding region of the active GAPDH, for a region located 1 kb upstream of the GAPDH promoter (cat. No. C17011003) and for the Sat2 satellite repeat. Figure 1 shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).

GAPDH promoter and for the coding region of the active ACTB gene (figure 2A). The IP'd DNA was subsequently analysed on an Illumina Genome Analyzer. Library preparation, cluster generation and sequencing were performed according to the manufacturer's instructions. The 36 bp tags were aligned to the human genome using the ELAND algorithm. Figure 2B shows the obtained profiles in genomic regions of chromosome 12 (including the GAPDH positive control), 7 (including the ACTB positive control), 14 and 3, respectively. These results clearly show an enrichment of the H3K36me3 at active genes.

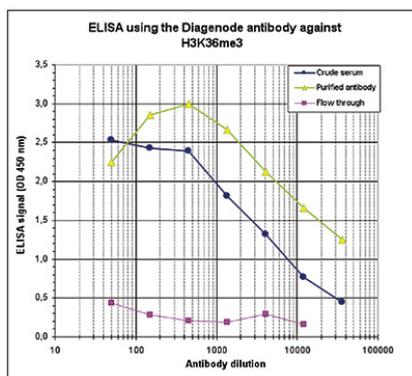


Figure 3. Determination of the antibody titer

To determine the titer of the antibody, an ELISA was performed using a serial dilution of the Diagenode antibody directed against H3K36me3 (cat. No. C15410058) 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 3), the titer of the purified antibody was estimated to be 1:19,300.

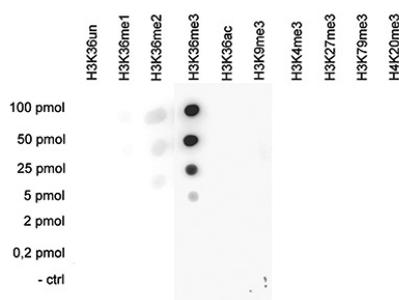


Figure 4. Cross reactivity tests using the Diagenode antibody directed against H3K36me3

A Dot Blot analysis was performed to test the cross reactivity of the Diagenode antibody against H3K36me3 (cat. No. C15410058) with peptides containing other H3 and H4 modifications 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 1:20,000. Figure 4 shows a high specificity of the antibody for the modification of interest.

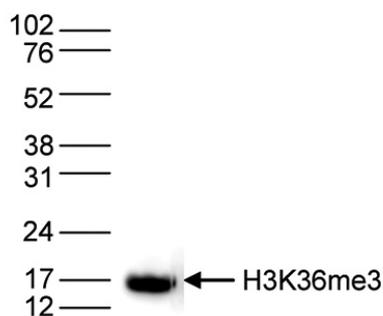


Figure 5. Western blot analysis using the Diagenode antibody directed against H3K36me3

Histone extracts (15 µg) from HeLa cells were analysed by Western blot using the Diagenode antibody directed against H3K36me3 (cat. No. C15410058) 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.