diagenode
A Hologic Company

TECHNICAL DATASHEET

H3K36me3 polyclonal antibody - Classic

Cat. No. C15310058

Type: Polyclonal	Specificity: Human, mouse: positive. Other species: not tested.
Size: 100 µl	Isotype: NA
Concentration: Not determined	Host: Rabbit
Lot No.: A114-001	Purity: Whole antiserum from rabbit containing 0.05% azide.
Storage buffer: NA	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 containing the trimethylated lysine 36 (H3K36me3), using a KLH-conjugated synthetic peptide.

Applications

Applications	Suggested dilution	References
ChIP *	5 - 10 µl/ChIP	Fig 1, 2
ELISA	1:100 - 1:500	Fig 3
Dot Blotting	1:100,000	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 1-10 µl 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 histone H3K36 is preferentially present at active genes.

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Validation Data

A.

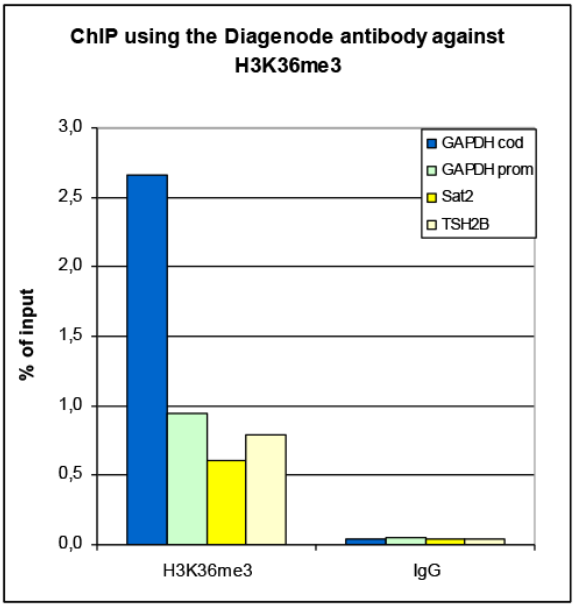
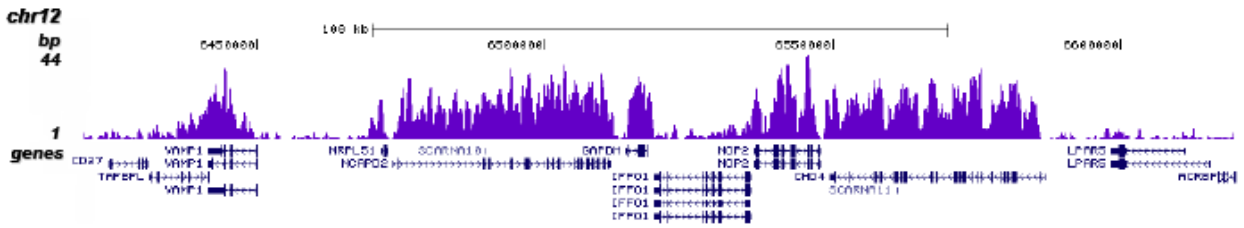


Figure 1. ChIP-seq results obtained with the Diagenode antibody directed against H3K36me3

ChIP was performed with 5 µl of the Diagenode antibody against H3K36me3 (cat. No. CS-058-050) on sheared chromatin from 1 million HeLaS3 cells using the "Auto Histone ChIP-seq" kit (cat. No. AB-Auto02-A100) on the IP-Star automated system. IgG (2 µg/IP) was used as a negative IP control. The IP'd DNA was analysed by QPCR with optimized PCR primer pairs for the coding and promoter region of the active GAPDH gene, for the coding region of the inactive TSH2B gene and for the Sat2 satellite repeat (figure 2A). The IP'd DNA was subsequently analysed with 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 results in 200 kb regions of chromosome 12 (including the GAPDH positive control), 6 and 7 and 14. These results clearly show an enrichment of the H3K36me3 at active genes

B.



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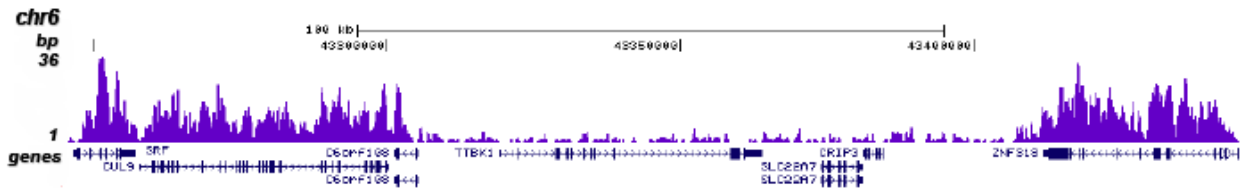

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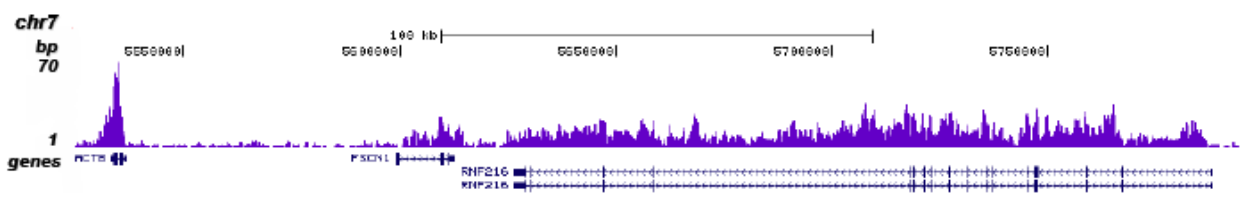

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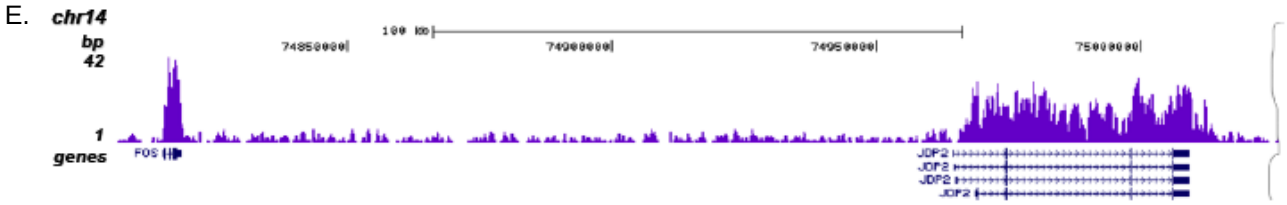

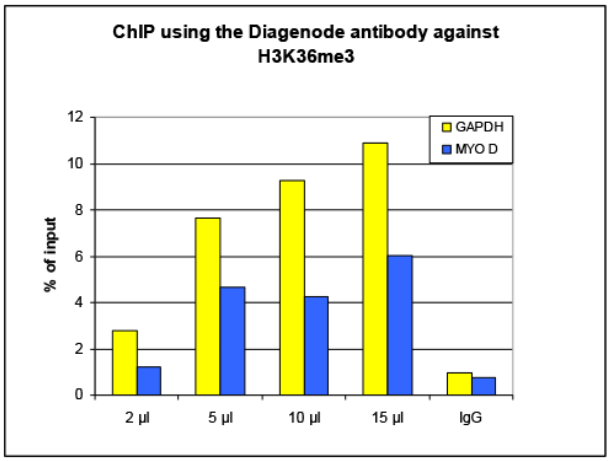

Figure 2. ChIP results obtained with the Diagenode antibody directed against H3K36me3

ChIP assays were performed using human osteosarcoma (U2OS) cells, the Diagenode antibody against H3K36me3 (cat. No. CS-058-100) 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 1.6 million cells. A titration of the antibody consisting of 2, 5, 10 and 15 µl per ChIP experiment was analysed. IgG (5 µg/IP) was used as a negative IP control. Quantitative PCR was performed using primer sets for the housekeeping gene GAPDH and for myogenic differentiation 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.

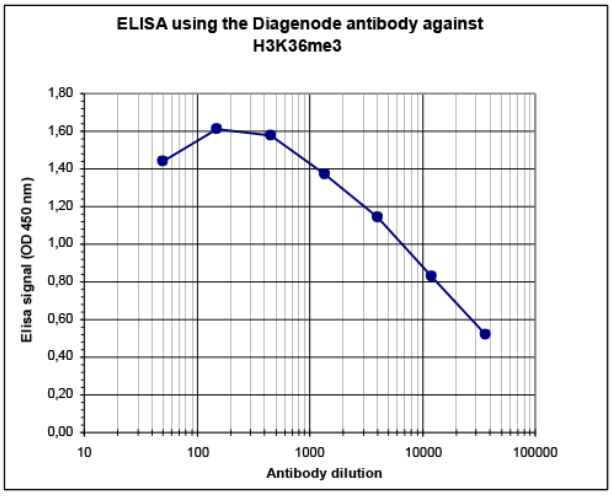

Figure 3. Determination of the titer

To determine the titer, an ELISA was performed using a serial dilution of the Diagenode antibody directed against H3K36me3 (cat. No. CS-058-100). 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 1:12,700.

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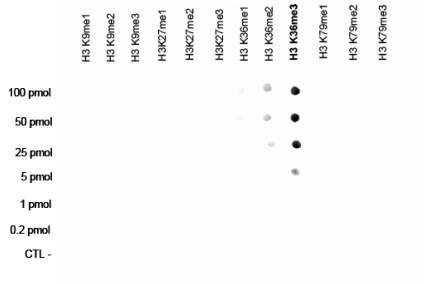

Figure 4. Cross reactivity test using the Diagenode antibody directed against H3K36me3

A Dot Blot analysis was performed to test the cross reactivity of the Diagenode antibody against H3K36me3 (cat. No. CS-058-050) with peptides containing other modifications of histone H3. Other histone modifications include mono- and dimethylation of the same lysine and mono-, di- and trimethylation of lysine 9, 27 and 79. 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 4 shows a high specificity of the antibody for the modification of interest.

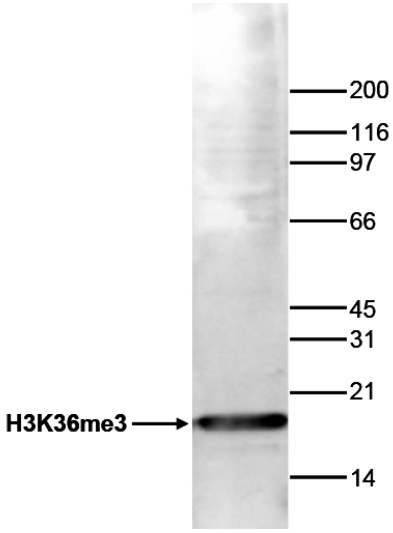

Figure 5. Western blot analysis using the Diagenode antibody directed against H3K36me3

Histone extracts (15 µg) from HeLa cells were analysed by Western blot using the Diagenode antibody against H3K36me3 (cat. No. CS-058-100) diluted 1:1,000 in TBS-Tween containing 5% skimmed milk. The position of the protein of interest is indicated on the left; the marker (in kDa) is shown on the right.

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