diagenode
A Hologic Company

TECHNICAL DATASHEET

H3K36me3 Antibody - ChIP-seq Grade

Cat. No. C15210013

Type: Monoclonal **ChIP-seq grade**	Specificity: Human, wide range expected.
Size: 100 µg	Isotype: NA
Concentration: 1 µg/µl	Host: Rabbit
Lot No.: 001	Purity: Affinity purified polyclonal antibody.
Storage buffer: PBS containing 50% glycerol, 1% BSA and 0.09% azide.	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.	

Last Data Sheet Update: August 10, 2020

Description

Monoclonal antibody raised in rabbit against the region of histone H3 containing the trimethylated lysine 36 (H3K36me3), using a KLH-conjugated synthetic peptide.

Applications

Applications	Suggested dilution	References
ChIP/ChIP-seq *	0.5 - 1 µg per IP	Fig 1, 2
Dot Blotting	1:2,000	Fig 3
Western Blotting	1:500	Fig 4

*Please note that the optimal antibody amount per IP should be determined by the end-user. We recommend testing 0.5 - 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. Methylation of histone H3K36 is associated with active genes.

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Validation data

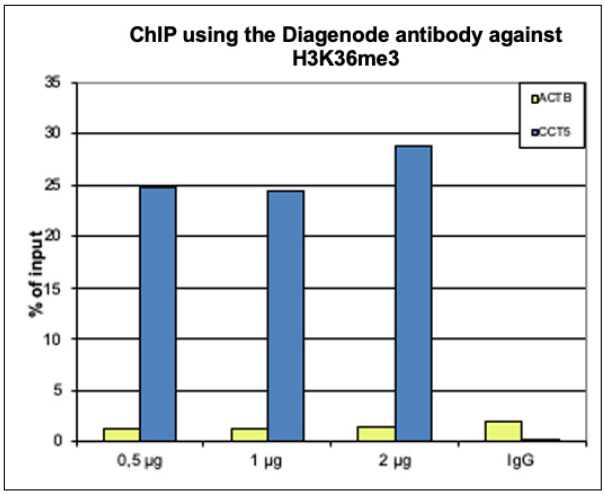

Figure 1. ChIP results obtained with the Diagenode monoclonal antibody directed against H3K36me3

ChIP was performed with the Diagenode antibody against H3K36me3 (cat. No. C15210013) on sheared chromatin from 500,000 HeLaS3 cells using the "iDeal ChIP-seq" kit (cat. No. C01010051). A titration of the antibody consisting of 0.5, 1, and 2 µg per ChIP experiment was analysed. IgG (1 µg/IP) was used as negative IP control. Quantitative PCR was performed with primers specific for the CCT5 coding region, used as positive control, and for a region upstream the ACTB promoter, used as negative control. The graph shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).

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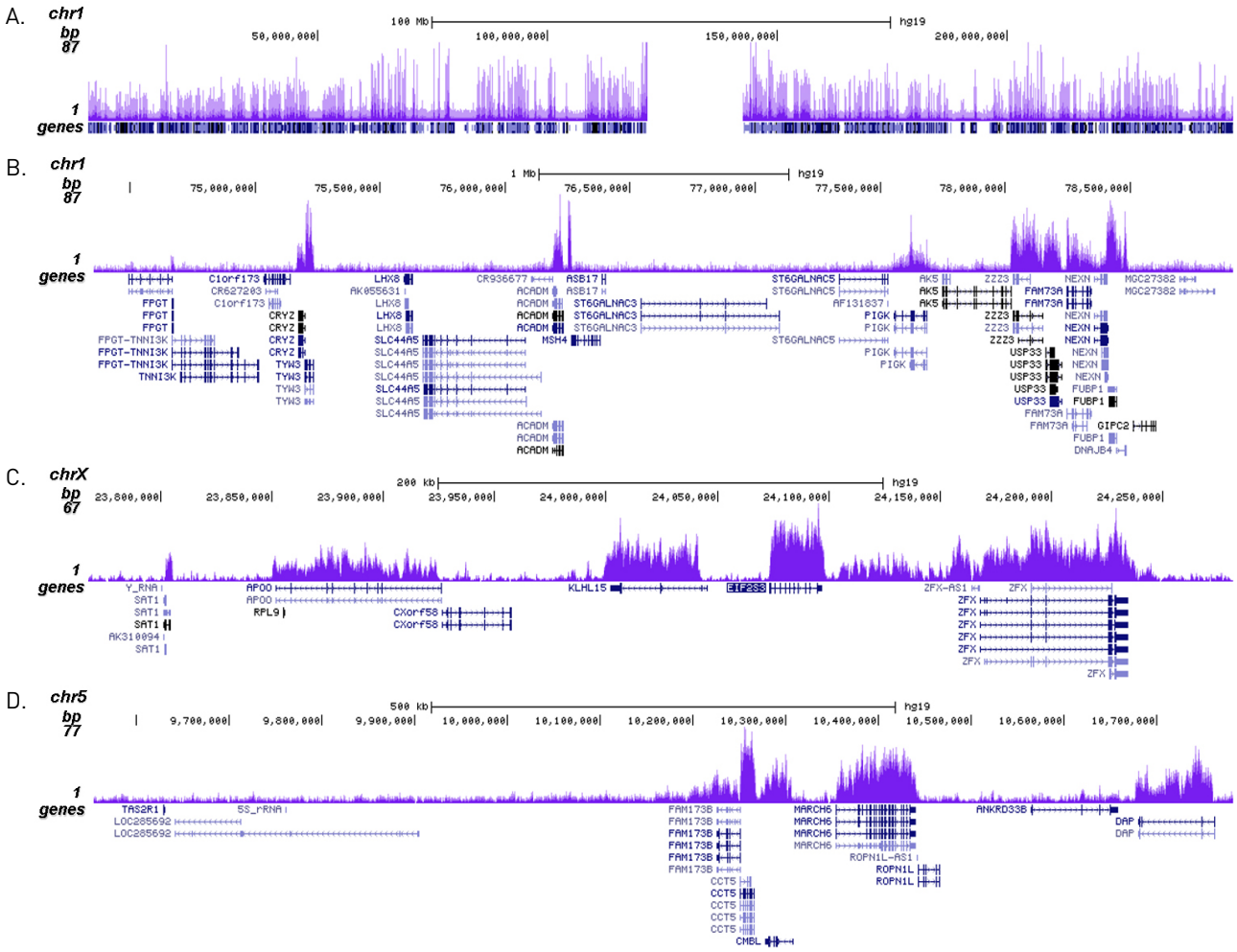

Figure 2. ChIP-seq results obtained with the Diagenode antibody directed against H3K36me3
ChIP was performed on sheared chromatin from 500,000 HeLaS3 cells using 1 µg of the Diagenode antibody against H3K36me3 (cat. No. C15210013) as described above. The IP'd DNA was subsequently analysed on an Illumina NovaSeq. Library preparation, cluster generation and sequencing were performed according to the manufacturer's instructions. The 51 bp tags were aligned to the human genome using the BWA algorithm. Figure 2 shows the H3K36me3 signal along the complete sequence and 1.5 Mb region of human chromosome 1 (figure 2A and B), in a genomic region on chromosome X surrounding the EIF2S3 gene (figure 2C) and in a genomic region surrounding the CCT5 positive control (figure 2D).

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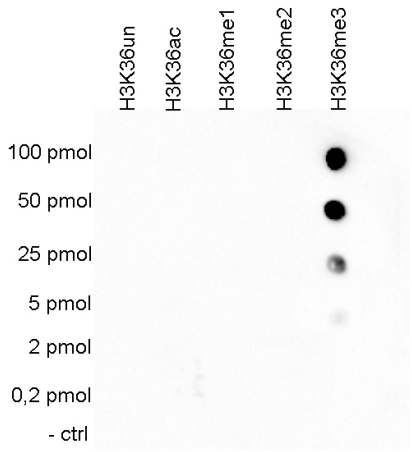

Figure 3. Cross reactivity tests using the Diagenode monoclonal antibody directed against H3K36me3
To test the cross reactivity of the Diagenode antibody against H3K36me3 (cat. No. C15210013), a Dot Blot analysis was performed with peptides containing other histone modifications and the unmodified H3K36. One hundred to 0.2 pmol of the respective peptides were spotted on a membrane. The antibody was used at a dilution of 1:2,000. Figure 2 shows a high specificity of the antibody for the modification of interest.

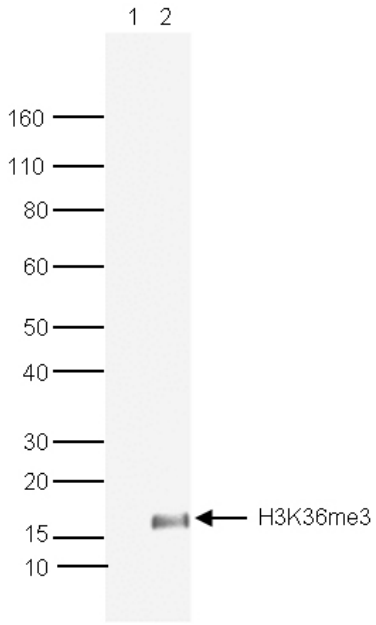

Figure 4. Western blot analysis using the Diagenode monoclonal antibody directed against H3K36me3
Western blot was performed on recombinant histone H3 (1 µg) and on histone extracts from HeLa cells (40 µg) using the Diagenode antibody against H3K36me3 (cat. No. C15210013). The antibody was diluted 1:500 in TBS-Tween containing 5% skimmed milk. The position of the protein of interest is shown on the right, the marker (in kDa) is shown on the left.

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