diagenode
A Hologic Company

ChIP-seq grade

TECHNICAL DATASHEET

H3K79me2 polyclonal antibody

Cat. No. C15410051
Type: Polyclonal **ChIP-grade/ChIP-seq-grade**
Source: Rabbit
Lot #: A1193D
Size: 50 µg/ 46 µl
Concentration: 1.1 µg/µl

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

Description: Polyclonal antibody raised in rabbit against the region of histone H3 containing the dimethylated lysine 79 (H3K79me2), using a KLH-conjugated synthetic peptide.

Applications

	Suggested dilution	References
ChIP*	1-2 µg/ChIP	Fig 1, 2
ELISA	1:500	Fig 3
Dot blotting	1:5,000	Fig 4
Western blotting	1:200	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.

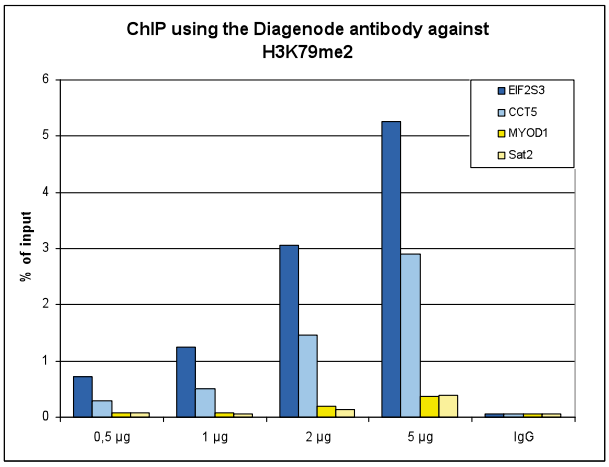

Figure 1. ChIP results obtained with the Diagenode antibody directed against H3K79me2

ChIP assays were performed using human HeLa cells, the Diagenode antibody against H3K79me2 (Cat. No. C15410051) and optimized PCR primer pairs for qPCR. ChIP was performed with the "Auto Histone ChIP-seq" kit (cat. No. C01010020), using sheared chromatin from 1 million cells. A titration consisting of 0.5, 1, 2 and 5 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 specific for the coding regions of the active EIF2S3 and CCT5 genes, used as positive controls, and for the inactive MYOD1) gene and the Sat2 satellite repeat, used as negative controls. Figure 1 shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).

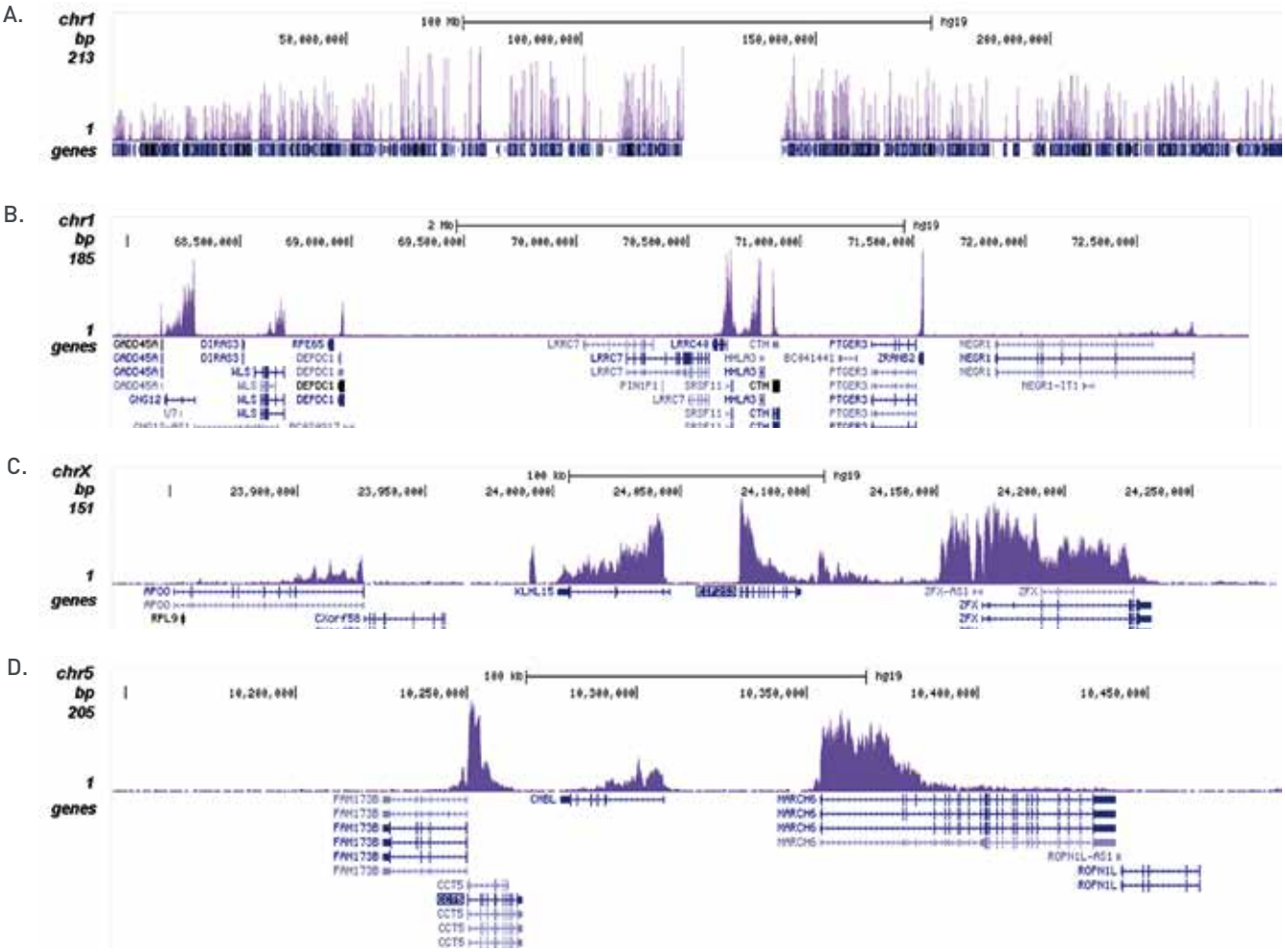

Figure 2. ChIP-seq results obtained with the Diagenode antibody directed against H3K79me2

ChIP was performed with 1 µg of the Diagenode antibody against H3K79me2 (Cat. No. C15410051) as described above. The IP'd DNA was subsequently analysed on an Illumina HiSeq 2000. Library preparation, cluster generation and sequencing were performed according to the manufacturer's instructions. The 50 bp tags were aligned to the human genome using the BWA algorithm. Figure 2 shows the peak distribution along the complete sequence and a 5 Mb region of chromosome 1 (figure 2A and B) and in two 300 kb regions surrounding the EIF2S3 and CCT5 positive control genes (figure 2C and D).

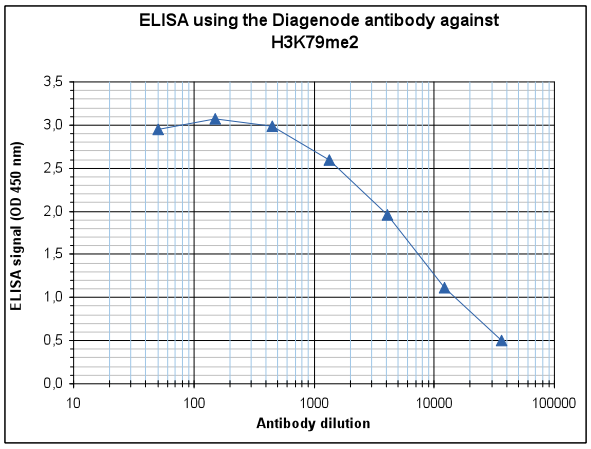

Figure 3. Determination of the antibody titer

To determine the titer of the antibody, an ELISA was performed using a serial dilution of the Diagenode antibody directed against H3K79me2 (Cat. No. C15410051). 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 1:6,600

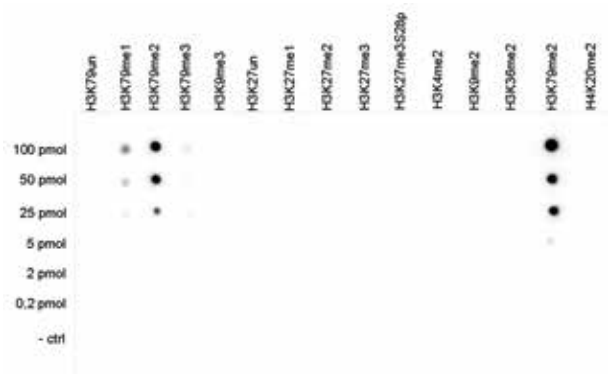

Figure 4. Cross reactivity tests using the Diagenode antibody directed against H3K79me2

A Dot Blot analysis was performed to test the cross reactivity of the Diagenode antibody against H3K79me2 (Cat. No. C15410051) with peptides containing other modifications and unmodified sequences of histone H3. 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 1:5,000. Figure 4 shows a high specificity of the antibody for the modification of interest.

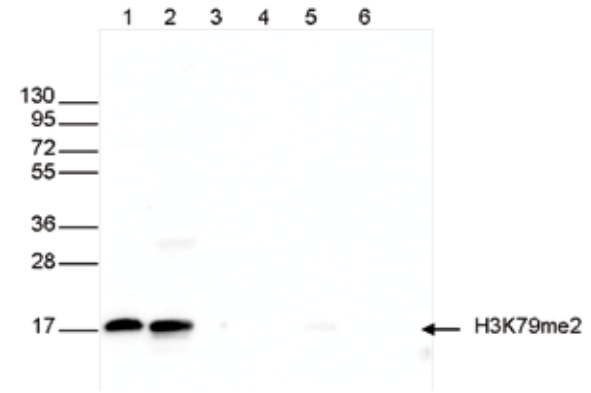

Figure 5. Western blot analysis using the Diagenode antibody directed against H3K79me2

Western blot was performed on whole cell (25 µg, lane 1) and histone extracts (15 µg, lane 2) from HeLa cells, and on 1 µg of recombinant histone H2A, H2B, H3 and H4 (lane 3, 4, 5 and 6, respectively) using the Diagenode antibody against H3K79me2 (Cat. No. C15410051) diluted 1:200 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.

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