TECHNICAL DATASHEET

LOT: A2014P

HOLOGIC® Diagenode

H3K79me3 antibody

Cat. No. C15410068

Lot:	A2014P
Size:	50 µg
Type:	Polyclonal, **ChIP/ChIP-seq grade**
Isotype:	NA
Source:	Rabbit
Concentration:	0.6 µg/µl

Specificity:	Human, mouse, yeast: positive Other species: not tested
Purity:	Affinity purified

Storage buffer: PPBS 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 histone H3 containing the trimethylated lysine 79 (H3K79me3), using a KLH-conjugated synthetic peptide.

Applications

Applications	Suggested dilution	References
ChIP*	1 – 2 µg per ChIP	Fig 1, 2
ELISA	1:500 – 1/1,000	Fig 3
Dot blotting	1:20,000	Fig 4
Western blotting	1:100	Fig 5

*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. Trimethylation of histone H3 on K79 was shown to be more present at active promotors than at silent promotors.

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Last update: November, 2024

Results

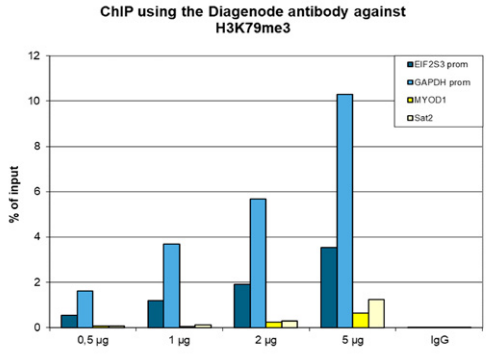

Figure 1: ChIP results obtained with the Diagenode antibody directed against H3K79me3

ChIP was performed with the Diagenode antibody against H3K79me3 (cat. No. C15410068) on sheared chromatin from 500,000 HeLa cells using the "iDeal ChIP-seq kit for histone (Cat. No. C01010051). A titration of the antibody consisting of 0.5, 1, 2 and 5 µg per ChIP experiment was analysed. IgG (2 µg/IP) was used as negative IP control. Quantitative PCR was performed with primers for the GAPDH and EIF2S3 promoters, used as positive control 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). These results are in accordance with the observation that H3K79me3 shows a preference for active genes.

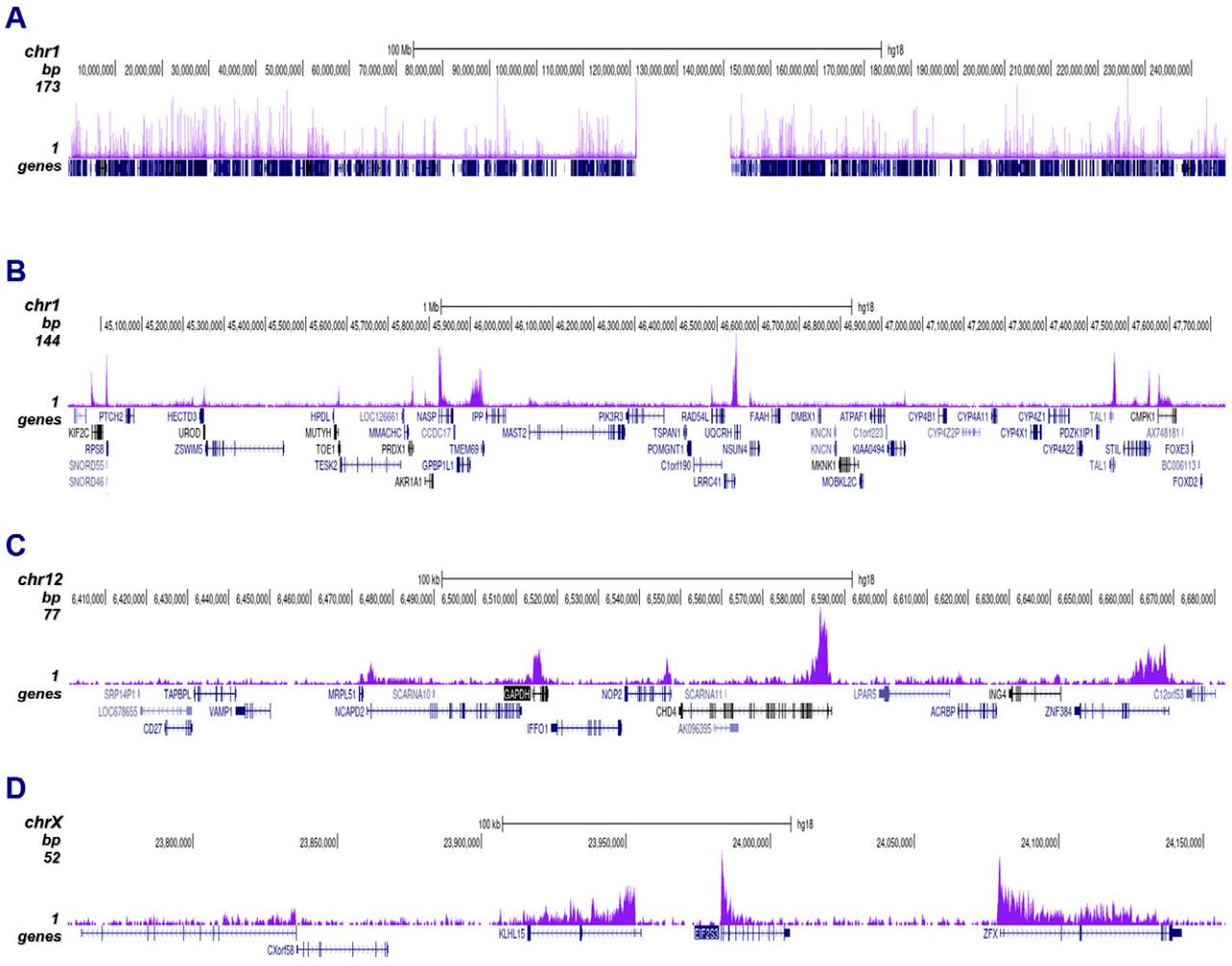

Figure 2: ChIP-seq results obtained with the Diagenode antibody directed against H3K79me3

ChIP was performed on 100,000 K562 cells using 1 µg of the Diagenode antibody against H3K79me3 (cat. No. C15410068). The IP'd DNA was 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 2 shows the peak distribution along the complete sequence and a 3 Mb region of human chromosome 1 (figure 2A and B), in a 2 genomic regions surrounding the GAPDH and EIF2S3 positive control genes (figure 2C and D).

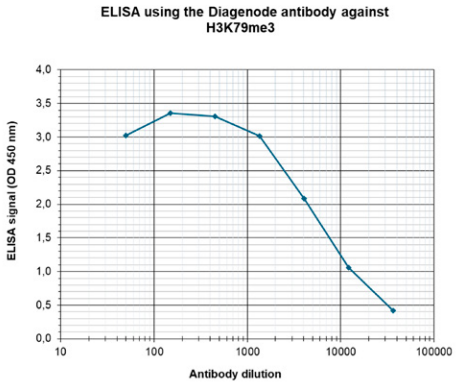

Figure 3: Determination of the antibody titer

To determine the titer of the antibody, an ELISA was performed using a serial dilution of Diagenode antibody directed against H3K79me3 (cat. No. C15410068) in antigen coated wells. 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 1:6,700.

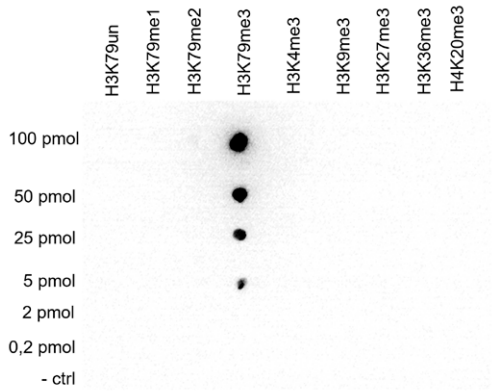

Figure 4: Cross reactivity tests using the Diagenode antibody directed against H3K79me3

A Dot Blot analysis was performed to test the cross reactivity of the Diagenode antibody against H3K79me3 (cat. No. C15410068) with peptides containing other histone modifications and the unmodified H3K79. One hundred to 0.2 pmol of the respective peptides 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.

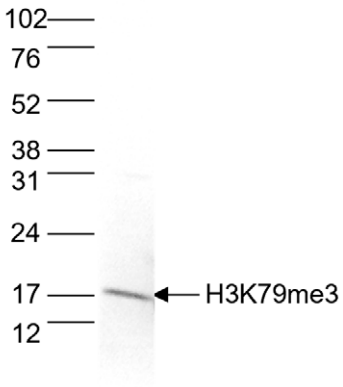

Figure 5: Western blot analysis using the Diagenode antibody directed against H3K79me3

Whole cell protein extracts from HeLa cells (25 µg) were analysed by Western blot using the Diagenode antibody against H3K79me3 (cat. No. C15410068), diluted 1:100 in TBS-Tween containing 5% skimmed milk. The molecular weight marker is shown on the right; the location of the protein of interest is indicated on the left.