TECHNICAL DATASHEET

LOT: A3236P

HOLOGIC® Diagenode

H3K27me3 antibody

Cat. No. C15410195

Lot:	A3236P
Size:	10 µg / 50 µg
Type:	Polyclonal, **ChIP-grade**, **ChIP-seq grade**, **CUT&Tag-grade**
Isotype:	NA
Source:	Rabbit
Concentration:	0.85 µg/µl

Specificity:	Human, mouse, rat, pig, cow, zebrafish, Drosophila, Schistosoma, Arabidopsis: positive. Other species: not tested.
Purity:	Affinity purified polyclonal antibody
Storage buffer:	PBS containing 0.05% azide

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 trimethylated lysine 27 (H3K27me3), using a KLH-conjugated synthetic peptide.

Applications

Applications	Suggested dilution	References
ChIP/ChIP-seq*	0.5–1 µg per IP	Fig 1, 2
CUT&Tag	1 µg	Fig 3
ELISA	1:500	Fig 4
Dot blotting	1:5,000	Fig 5
Western blotting	1:1,000	Fig 6
Immunofluorescence	1:200	Fig 7

*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 H3K27 is associated with inactive genomic regions.

Diagenode SA
BELGIUM | EUROPE
LIEGE SCIENCE PARK
Rue du Bois Saint-Jean, 3
4102 Seraing - Belgium
Tel: +32 4 364 20 50
Fax: +32 4 364 20 51
orders.diagenode@hologic.com
support.diagenode@hologic.com

Diagenode LLC
USA | NORTH AMERICA
400 Morris Avenue, Suite 101
Denville, NJ 07834 - USA
Tel: +1 862 209-4680
Fax: +1 862 209-4681
orders.na@diagenode.com
info.na@diagenode.com

Last update: September, 2025

Results

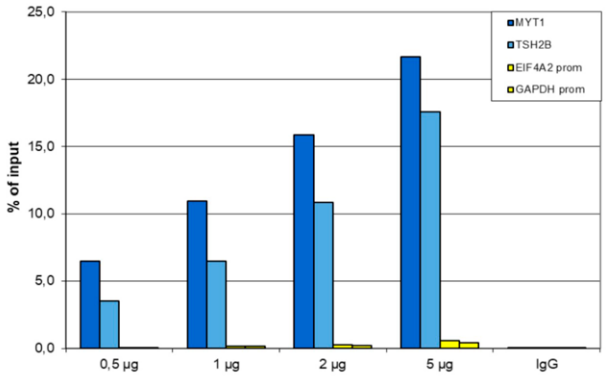

Figure 1: ChIP results obtained with the antibody directed against H3K27me3

ChIP assays were performed using human HeLa cells, the antibody against H3K27me3 (cat. No. C15410195) and optimized PCR primer pairs for qPCR. ChIP was performed with the "iDeal ChIP-seq" kit (cat. No. C01010051) on sheared chromatin from 500,000 cells, using 0.5, 1, 2 and 5 µg of antibody per ChIP experiment. IgG (1 µg/IP) was used as a negative IP control. Quantitative PCR was performed with primers specific for the promoter of the active genes GAPDH and EIF4A2, used as negative controls, and TSH2B and MYT1, used as positive controls. The figure shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis)

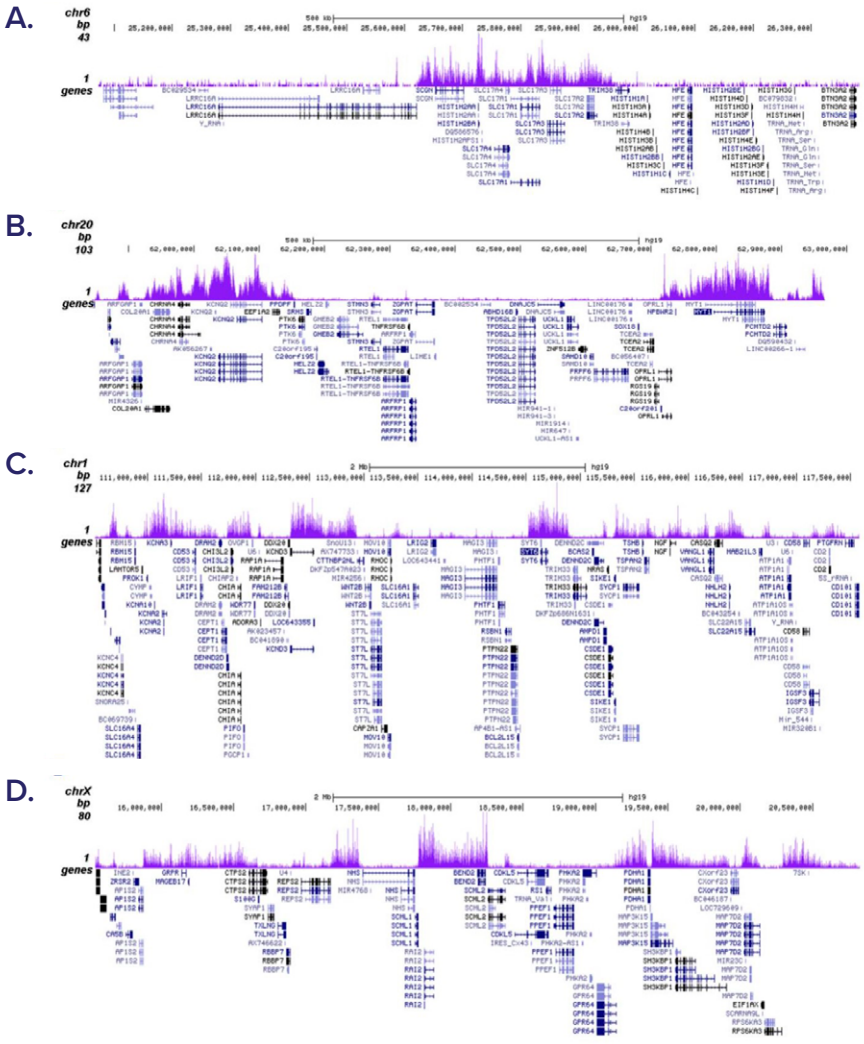

Figure 2: ChIP-seq results obtained with the antibody directed against H3K27me3

ChIP was performed on sheared chromatin from 500,000 HeLa cells using 0.5 µg of the antibody against H3K27me3 (cat. No. C15410195) as described above. The IP'd DNA was subsequently analysed on an Illumina NovaSeq (2x50 bp paired-end reads) according to the manufacturer's instructions. The tags were aligned to the human genome (hg38) using the BWA algorithm. Figure 2 shows the enrichment in genomic regions of chromosome 6 and 20, surrounding the TSH2B and MYT1 positive control genes (fig 2A and 2B, respectively), and in two genomic regions of chromosome 1 and X (figure 2C and D).

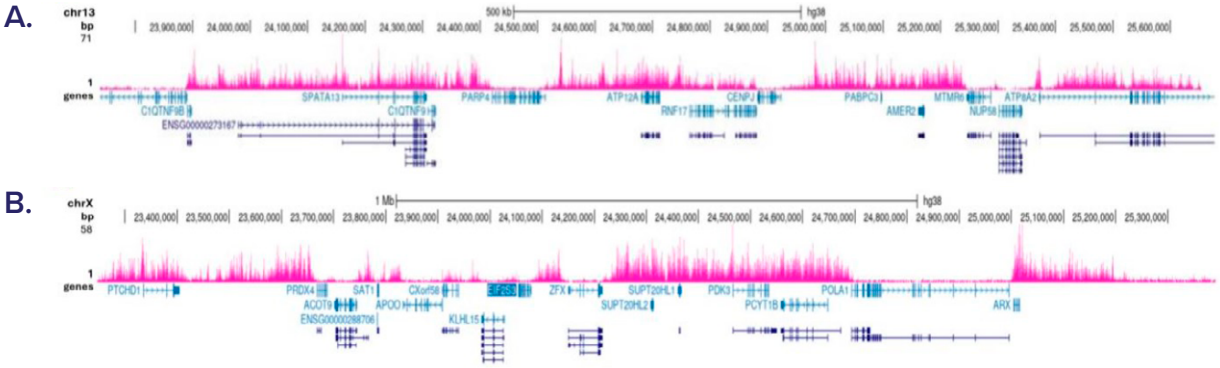

Figure 3: Cut&Tag results obtained with the antibody directed against H3K27me3

Cut&Tag was performed on 50,000 K562 cells using 1 µg of the antibody against H3K27me3 (cat. No. C15410195) and the Diagenode pA-Tn5 transposase (C01070001). The libraries were subsequently analysed on an Illumina NovaSeq sequencer (2x50 bp paired-end reads) according to the manufacturer's instructions. The tags were aligned to the human genome (hg38) using the BWA algorithm. Figure 3 shows the peak distribution in 2 genomic regions on chromosome 13 and the X-chromosome (figure 3A and B, respectively).

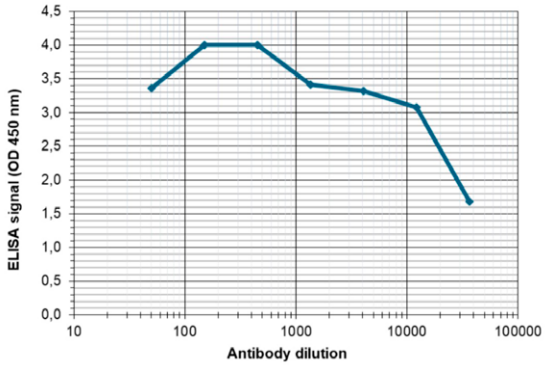

Figure 4: Determination of the antibody titer

To determine the titer of the antibody, an ELISA was performed using a serial dilution of the antibody against H3K27me3 (cat. No. C15410195). The antigen used was a peptide containing the histone modification of interest. By plotting the absorbance against the antibody dilution (Figure 4), the titer of the antibody was estimated to be 1:30,000.

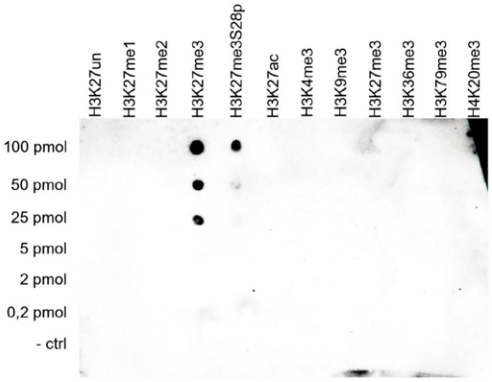

Figure 5: Cross reactivity tests using the antibody directed against H3K27me3

To test the cross reactivity of the antibody against H3K27me3 (cat. No. C15410195), a Dot Blot analysis was performed with peptides containing other modifications or unmodified sequences of histone H3 and H4. One hundred to 0.2 pmol of the respective peptides were spotted on a membrane. The antibody was used at a dilution of 1:5,000. Figure 5 shows a high specificity of the antibody for the modification of interest.

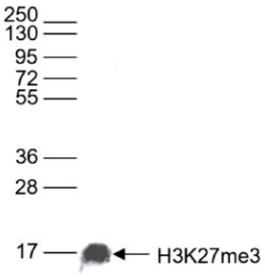

Figure 6: Western blot analysis using the antibody directed against H3K27me3

Western blot was performed on whole cell lysates (25 µg)) using the Diagenode antibody against H3K27me3 (cat. No. C15410195). The antibody was diluted 1:1,000 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.

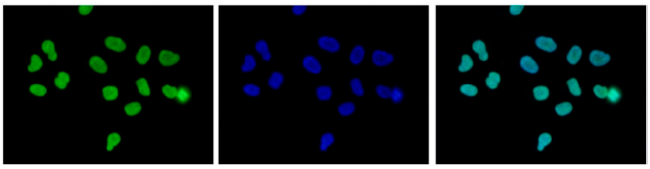

Figure 7: Immunofluorescence using the antibody directed against H3K27me3

Human HeLa cells were stained with the antibody against H3K27me3 (cat. No. C15410195) and with DAPI. Cells were fixed with 4% formaldehyde for 10' and blocked with PBS/TX-100 containing 5% normal goat serum and 1% BSA. The cells were immunofluorescently labelled with the H3K27me3 antibody (left) diluted 1:200 in blocking solution followed by an anti-rabbit antibody conjugated to Alexa488. The middle panel shows staining of the nuclei with DAPI. A merge of the two stainings is shown on the right.