diagenode
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TECHNICAL DATASHEET

# H3K27me3 antibody

## Cat. No. C15200181

**Type:** Monoclonal, **ChIp grade**
**Isotype:** IgG1
**Source:** Mouse
**Lot:** 001-13
**Size:** 50 µg
**Concentration:** 1 µg/µl

**Specificity:** Human, Nematodes, Magnaporthe oryzae: positive. Other species: not tested.
**Purity:** Protein A purified monoclonal antibody.
**Storage:** Store at -20°C; for long storage, store at -80°C. Avoid multiple freeze-thaw cycles.
**Storage buffer:** PBS containing 0.05% azide.

**Precautions:** This product is for research use only. Not for use in diagnostic or therapeutic procedures.

**Description:** Monoclonal antibody raised in mouse against histone H3 trimethylated at lysine 27 (H3K27me3), using a KLH-conjugated synthetic peptide.

## Applications

| Applications | Suggested dilution | References |
|---|---|---|
| ChIP* | 1 - 2 µg per IP | Fig 1 |
| CUT&TAG | 1 µg | Fig 2 |
| ELISA | 1:3,000 | Fig 3 |
| Western blotting | 1:1,000 | Fig 4 |
| Immunofluorescence | 1:500 | 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.

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**Last update:** September, 2021

## Results

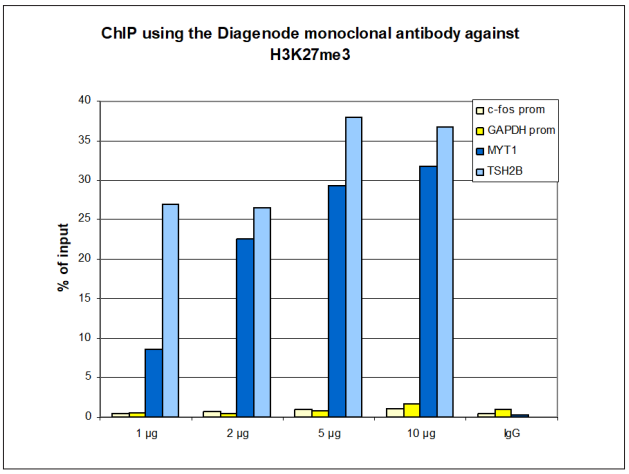


**Figure 1. ChIP results obtained with the Diagenode monoclonal antibody directed against H3K27me3**

ChIP assays were performed using human HeLa cells, the Diagenode monoclonal antibody against H3K27me3 (cat. No. C15200181) and optimized PCR primer sets for qPCR. ChIP was performed with the "AutoHistone ChIP-seq" kit (cat. No. C01010022) on sheared chromatin from 1 million cells. A titration of the antibody consisting of 1, 2, 5, and 10 µg per ChIP experiment was analysed. IgG (2 µg/IP) was used as negative IP control. QPCR was performed with primers for the promoters of the active genes c-fos (cat. No. C17011004) and GAPDH as negative controls, and for the coding regions of the inactive genes MYT1 and TSH2B (cat. No. C17011041) as positive 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 H3K27me3 is preferably present at inactive genes.

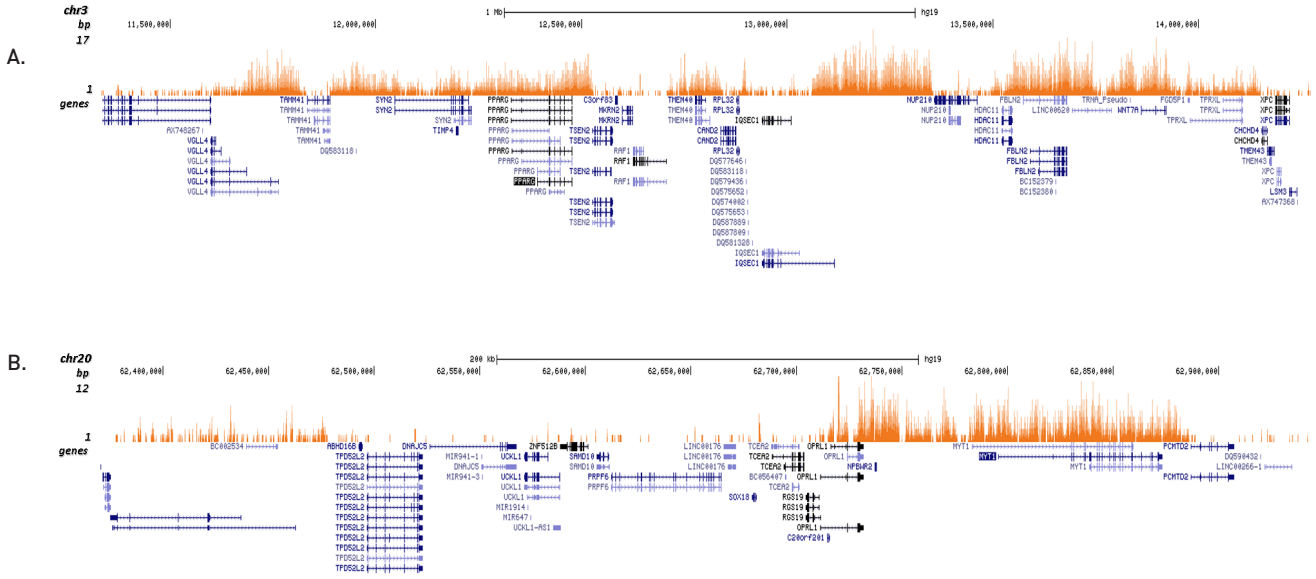


**Figure 2. Cut&Tag results obtained with the Diagenode monoclonal antibody directed against H3K27me3**

CUT&TAG (Kaya-Okur, H.S., Nat Commun 10, 1930, 2019) was performed on 50,000 K562 cells using 1 µg of the Diagenode monoclonal antibody against H3K27me3 (cat. No. C15200181) and the Diagenode pA-Tn5 transposase (C01070001). The libraries were subsequently analysed on an Illumina NextSeq 500 sequencer (2x75 paired-end reads) according to the manufacturer's instructions. The tags were aligned to the human genome (hg19) using the BWA algorithm. Figure 3 shows the peak distribution in 2 genomic regions on chromosome 3 and 20 (figure 2A and B, respectively).

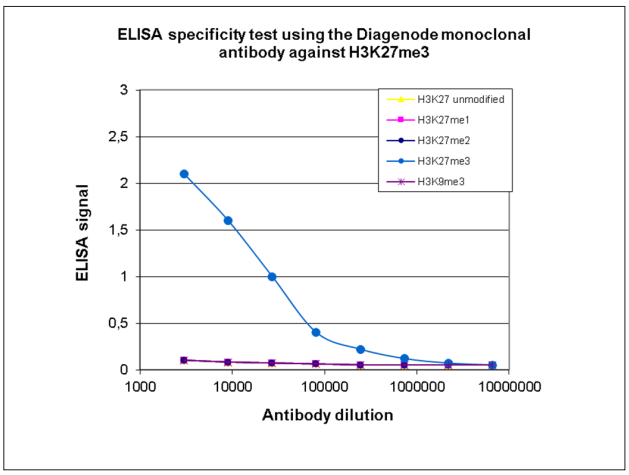


**Figure 3. Cross reactivity of the Diagenode monoclonal antibody directed against H3K27me3**

To test the specificity an ELISA was performed using a serial dilution of the Diagenode monoclonal antibody against H3K27me3 (cat. No. C15200181). The wells were coated with peptides containing the unmodified H3K27 region as well as the mono-, di- and trimethylated H3K27 and the trimethylated H3K9. Figure 3 shows a high specificity of the antibody for the peptide containing the modification of interest.

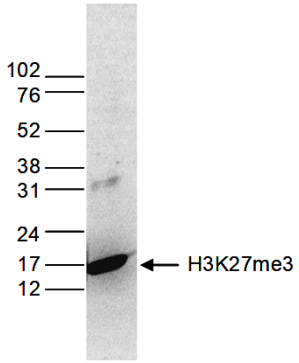


**Figure 4. Western blot analysis using the Diagenode monoclonal antibody directed against H3K27me3**

Histone extracts (15 µg) from HeLa cells were analysed by Western blot using the Diagenode monoclonal antibody against H3K27me3 (cat. No. C15200181) diluted 1:1,000 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.

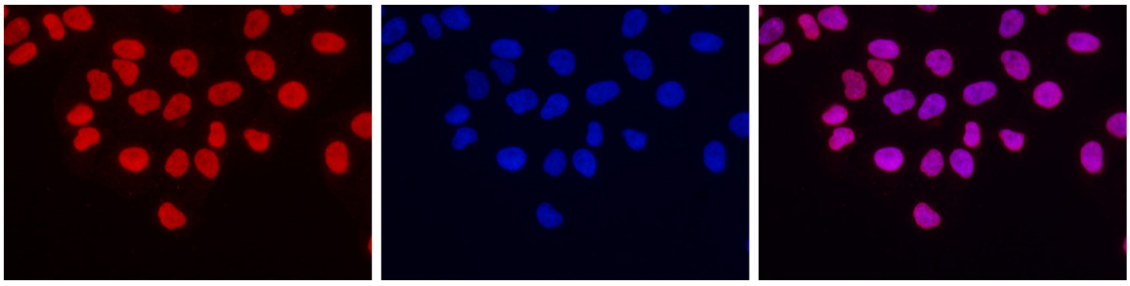

**Figure 5. Immunofluorescence using the Diagenode monoclonal antibody directed against H3K27me3**

HeLa cells were stained with the Diagenode antibody against H3K27me3 (cat. No. C15200181) 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 1:500 in blocking solution followed by an anti-mouse antibody conjugated to Alexa594. The middle panel shows staining of the nuclei with DAPI. A merge of the two stainings is shown on the right.