TECHNICAL DATA SHEET

| PRODUCT NAME<br>H3K9me3 polyclonal antibody | | |
|---|---|---|
| **Cat. No.** CS-056-100 | **Type:** Polyclonal **ChIP-grade** | **Size:** 100 µl |
| **Lot #:** A92-001 | **Source:** Rabbit | **Concentration:** not determined |

**Product description:** Polyclonal antibody raised in rabbit against histone H3 containing the trimethylated lysine 9 (H3K9me3), using a KLH-conjugated synthetic peptide.

**Specificity:** Human and mouse: positive
Other species: not tested

| Applications | Suggested dilution | References |
|---|---|---|
| ChIP | 1:5,000 | Fig 1 |
| ELISA | 1:1,000 | Fig 2 |
| Dot blotting | 1:10,000 | Fig 3 |
| Western blotting | 1:750 | Fig 4 |
| Immunofluorescence | 1:200 | Fig 5 |

**Purity:** Whole antiserum from rabbit 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.

**Last data sheet update:** April 22 , 2011

**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 H3K9 is associated with heterochromatin formation and gene silencing.

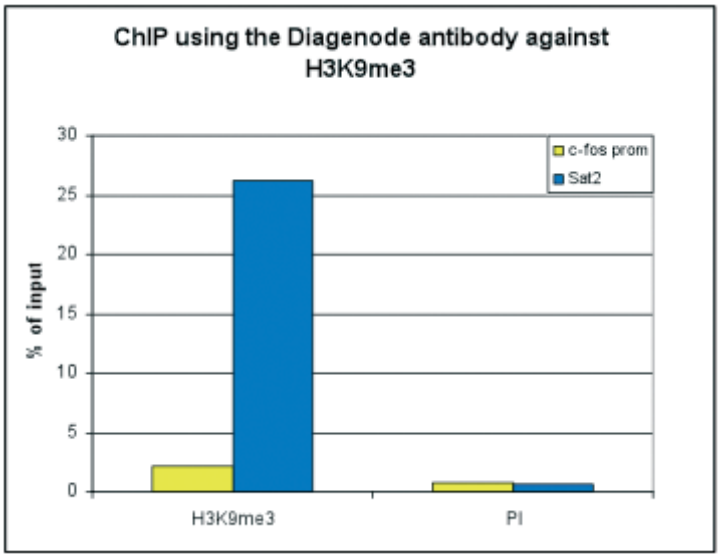


**Figure 1**

**ChIP results obtained with the Diagenode antibody directed against H3K9me3**

ChIP assays were performed using undifferentiated human teratocarcinoma cells (NCCIT), the Diagenode antibody against H3K9me3 (cat. No. CS-056-100) and optimized PCR primer sets for qPCR. Sheared chromatin from 10,000 cells was used per ChIP experiment. The antibody was diluted 1:5000. The pre-immune serum (PI, diluted 1:5000) was used as a negative control. Quantitative PCR was performed using primer sets for the satellite repeat Sat2 as a positive control and for the promoter of the house keeping gene c-fos, as a negative control. 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 H3K9me3 is preferably present at heterochromatin.

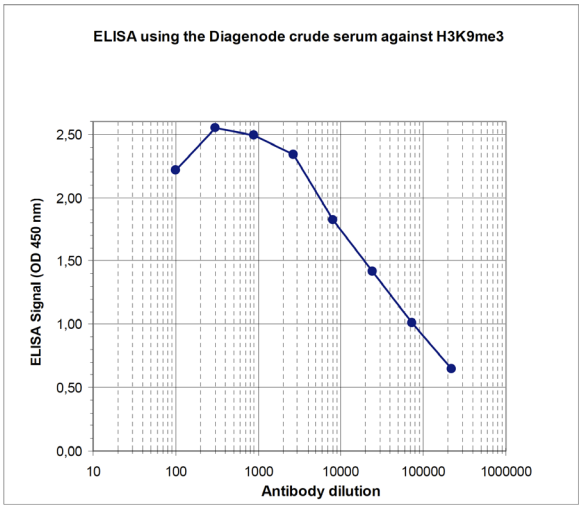


**Figure 2**

**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 H3K9me3 (cat. No. CS-056-100). The antigen used was a peptide containing the histone modification of interest. By plotting the absorbance against the antibody dilution (Figure 2), the titer of the antibody was estimated to be 1:35,000.

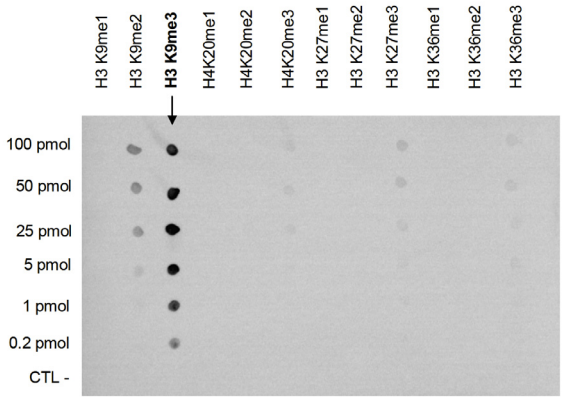


**Figure 3**

**Cross reactivity test using the Diagenode antibody directed against H3K9me3**

A Dot Blot analysis was performed to test the cross reactivity of the Diagenode antibody against H3K9me3 (cat. No. CS-056-100) with peptides containing other histone modifications of histone H3 and H4. Other histone modifications include mono- and dimethylation of the same lysine and mono-, di- and trimethylation of lysine 27 and 36 of H3, and of lysine 20 of H4. 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 1:10,000. Figure 3 shows a high specificity of the antibody for the modification of interest.

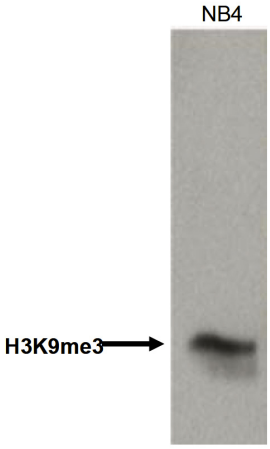


**Figure 4**
**Western blot analysis using the Diagenode antibody directed against H3K9me3**
Histone (acid) extracts of NB4 (human promyelocytic leukemia) cells were analysed by Western blot using the Diagenode antibody against H3K9me3 (cat. No. CS-056-100) diluted 1:750 in TBS-Tween containing 5% skimmed milk. The position of the protein of interest of interest is indicated on the left.

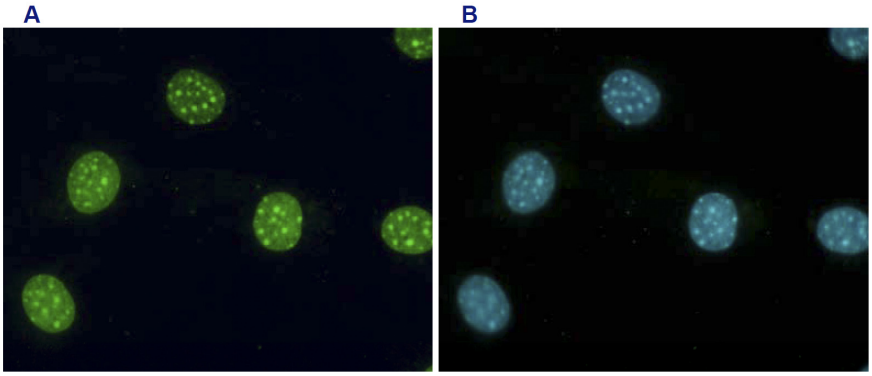


**Figure 5**
**Immunofluorescence analysis using the Diagenode antibody directed against H3K9me3**
NIH3T3 cells (mouse fibroblasts) were stained with the antibody against H3K9me3 (cat. No. CS-056-100) and with DAPI. Cells were formaldehyde fixed, permeabilized with TritonX100 and blocked with PBS containing 2.5% BSA.
Figure 5A: cells were immunofluorescently labelled with the H3K9me3 antibody (diluted 1:200 and incubated for 1 hour at room temperature) followed by goat anti-rabbit antibody conjugated to FITC.
Figure 5B: staining of the nuclei with DAPI, which specifically labels DNA.

Both antibody and DAPI staining are restricted to the nucleus. The dense signals obtained with both stainings characterize the distribution pattern of H3K9me3, which is linked to the transcriptionally inactive, condensed pericentric heterochromatin.