diagenode
A Hologic Company

TECHNICAL DATASHEET

PRODUCT NAME H3K9me3 monoclonal antibody		
Cat. No. SN-146-100	**Type:** Monoclonal **ChIP-grade**	**Size:** 100 µl
Lot #: 001	**Source:** Mouse	**Concentration:** not determined

Product description: Monoclonal antibody raised in mouse against histone H3 trimethylated at lysine 9 (H3K9me3), using a KLH-conjugated synthetic peptide.

Specificity: Human: positive
Other species: not tested

Applications	Suggested dilution	References
ChIP*	3 µl/ChIP	Fig 1
Dot blotting	1:10,000	Fig 2
Western blotting	1:1,000	Fig 3

*Please note that of the optimal antibody amount per IP should be determined by the end-user. We recommend testing 1-10 µl per IP.

Purity: Concentrated supernatant from a mouse hybridoma cell culture 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 29, 2011

Target description
Histones are present in the 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 H3K9 is associated with gene repression.

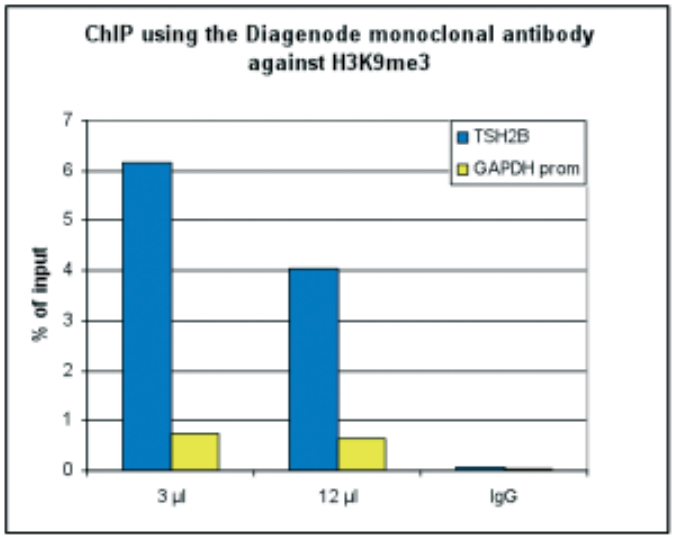

Figure 1

ChIP results obtained with the Diagenode monoclonal antibody directed against HDAC3

ChIP assays were performed using human HeLa cells, the Diagenode monclonal antibody against H3K9me3 (cat. No. SN-146-100) and optimized PCR primer sets for qPCR. ChIP was performed with the "LowCell# ChIP" kit (cat. No. kch-maglow-016), using sheared chromatin from 10,000 cells. Two different quantities of antibody (3 and 12 µl per ChIP experiment) were analysed. IgG (1 µg/IP) was used as negative IP control. QPCR was performed with primers for the GAPDH promoter and for the inactive gene TSH2B. Figure 1 shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).

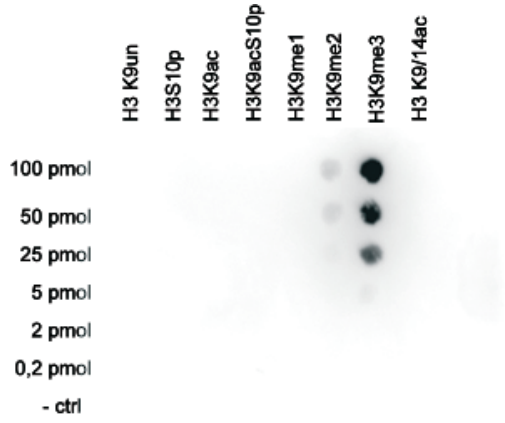

Figure 2

Cross reactivity tests using the Diagenode monoclonal antibody directed against H3K9me3

A Dot Blot analysis was performed to test the cross reactivity of the Diagenode monoclonal antibody against H3K9me3 (cat. No. SN-146-100) with peptides containing different modifications or 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 1:10,000. Figure 2 shows a high specificity of the antibody for the modification of interest.

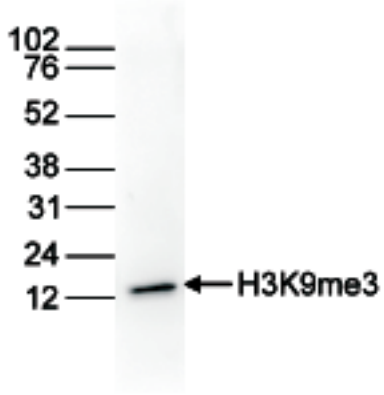

Figure 3

Western blot analysis using the Diagenode monoclonal antibody directed against H3K9me3

Histone extracts of HeLa cells (15 µg) were analysed by Western blot using the Diagenode monoclonal antibody against H3K9me3 (cat. No. SN-146-100) 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.