diagenode
A Hologic Company

TECHNICAL DATASHEET

H3K9me2 monoclonal antibody

Cat. No. C15200154

Type: Monoclonal	Specificity: Human, zebrafish, Arabidopsis, silena latifolia: positive. Other species: not tested.
Size: 50 µg	Isotype: IgG1
Concentration: 1.0 µg/µl	Host: Mouse
Lot No.: 001	Purity: Protein A purified monoclonal antibody.
Storage buffer: PBS containing 0.05% azide and 0.05% ProClin 300.	Storage conditions: 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: June 17, 2020

Description

Monoclonal antibody raised in mouse against histone H3, dimethylated at lysine 9 (H3K9me2), using a KLH-conjugated synthetic peptide.

Applications

Applications	Suggested dilution	References
ChIP *	3 µg/ChIP	Fig 1
ELISA	1:1,000	Fig 2
Western Blotting	1:1,000	Fig 3
Immunofluorescence	1:500	Fig 4

* 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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Validation Data

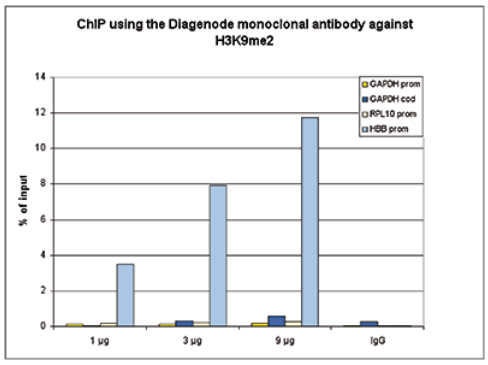

Figure 1. ChIP results obtained with the Diagenode monoclonal antibody directed against H3K9me2

ChIP assays were performed using HeLa cells, the monoclonal antibody against H3K9me2 (Cat. No. C15200154) and optimized PCR primer sets for qPCR. Chromatin was sheared with the Diagenode Bioruptor using the "Shearing ChIP" kit (Cat. No. C01020021). ChIP was performed with the "OneDay ChIP" kit (Cat. No. C01010080), using sheared chromatin from 1.6 million cells. A titration of the antibody consisting of 1, 3 and 9 µg per ChIP experiment was analysed. IgG (5 µg/IP) was used as negative IP control. QPCR was performed with primers for the promoter and the coding region of the GAPDH gene, and for the RPL10 and HBB promoters. Figure 1 shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).

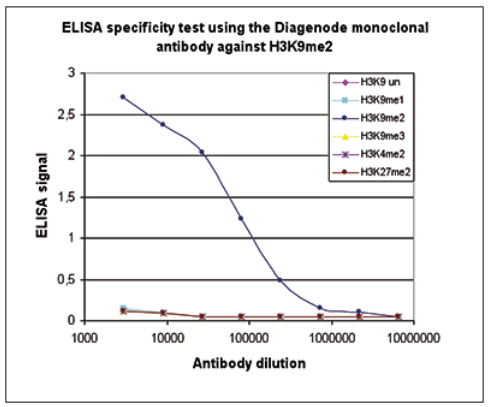

Figure 2. Cross reactivity of the Diagenode monoclonal antibody directed against H3K9me2

To test the specificity an ELISA was performed using a serial dilution of the Diagenode monoclonal antibody against H3K9me2 (Cat. No. C15200154). The wells were coated with peptides containing the unmodified H3K9 as well as the mono-, di- and trimethylated H3K9 and the dimethylated H3K4 and H3K27. 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 H3K9me2

Histone extracts (15 µg) from HeLa cells were analysed by Western blot using the Diagenode monoclonal antibody against H3K9me2 (Cat. No. C15200154) 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.

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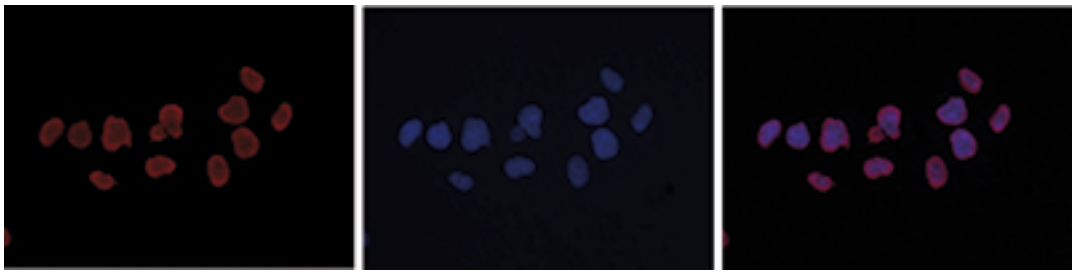

Figure 4. Immunofluorescence using the Diagenode monoclonal antibody directed against H3K9me2
HeLa cells were stained with the Diagenode antibody against H3K9me2 (Cat. No. C15200154) and with DAPI. Cells were fixed with 4% formaldehyde for 10' and blocked with PBS/TX-100 containing 5% normal goat serum and 1% BSA. The cells were immunofluorescently labelled with the H3K9me2 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.

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