diagenode
A Hologic Company

TECHNICAL DATASHEET

H4K20me2 monoclonal antibody

Cat. No. C15200220

Type: Monoclonal **ChIP-grade**

Source: Mouse

Lot #: 001-11

Size: 50 µg/ 50 µl

Concentration: 1 µg/µl

Specificity: Human: positive
Other species: not tested

Purity: Protein A purified monoclonal antibody in 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: Monoclonal antibody raised in mouse against histone H4, dimethylated at lysine 20 (H4K20me2), using a KLH-conjugated synthetic peptide.

Applications

	Suggested dilution	Results
ChIP*	1-5 µg/ChIP	Fig 1
Dot blot	1:20,000	Fig 2
Western blotting	1:1,000	Fig 3
IF	1:1,000	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. Dimethylation of H4K20 is associated with inactive genomic regions.

Results

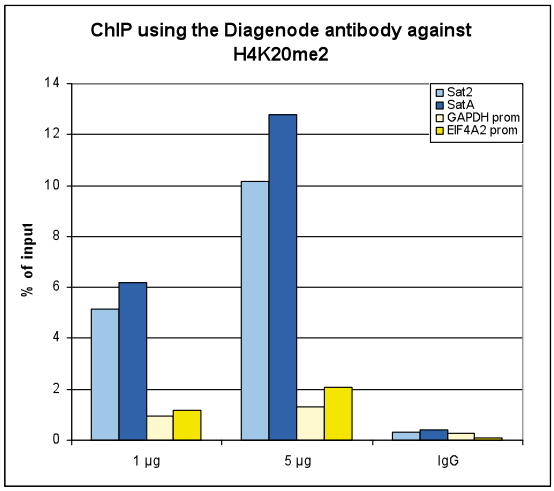

Figure 1. ChIP results obtained with the Diagenode monoclonal antibody directed against H4K20me2

ChIP assays were performed using HeLa cells, the monoclonal antibody against H4K20me2 (Cat. No. C15200220) and optimized PCR primer sets for qPCR. ChIP was performed with the Auto Histone ChIP-seq kit (Cat. No. C01010022) on the IP-Star automated system, using sheared chromatin from 1 million cells. Respectively 1 and 5 µg of antibody was used per ChIP experiment. IgG (1 µg/IP) was used as negative IP control. QPCR was performed with primers for the promoters of the GAPDH and EIF4A2 genes, used as negative controls, and for the Sat2 and SatA satellite repeats, used 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).

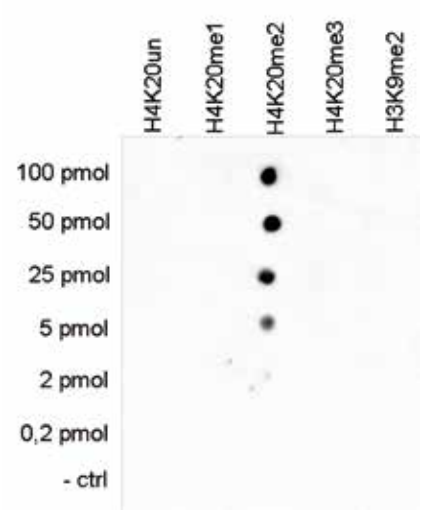

Figure 2. Cross reactivity tests using the Diagenode monoclonal antibody directed against H4K20me2

To test the cross reactivity of the Diagenode monoclonal antibody against H4K20me2 (Cat. No. C15200220), a Dot Blot analysis was performed with peptides containing other histone modifications and the unmodified H4K20. One hundred to 0.2 pmol of the respective peptides were spotted on a membrane. The antibody was used at a dilution of 1:20,000. Figure 2 shows a high specificity of the antibody for the modification of interest.

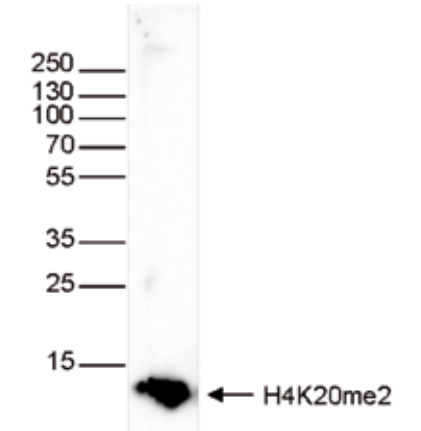

Figure 3. Western blot analysis using the Diagenode monoclonal directed antibody against H4K20me2

Histone extracts (15 µg) from HeLa cells were analysed by Western blot using the Diagenode monoclonal antibody against H4K20me2 (Cat. No. C15200220) 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.

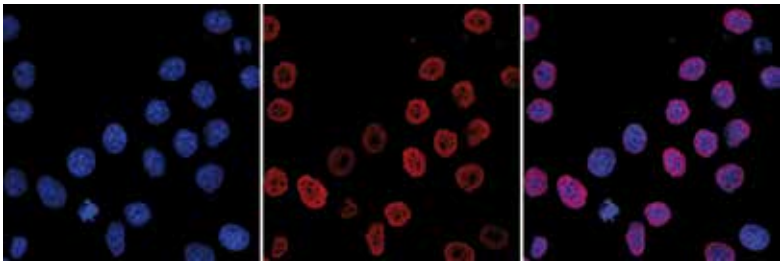

Figure 4. Immunofluorescence using the Diagenode monoclonal antibody directed against H4K20me2

HeLa cells were stained with the Diagenode monoclonal antibody against H4K20me2 (Cat. No. C15200220) and with DAPI. Cells were fixed with 4% formaldehyde for 10' and blocked with PBS/TX-100 containing 1% BSA. The cells were immunofluorescently labeled with the H4K20me2 antibody (middle) diluted 1:1.000 in blocking solution followed by an anti-mouse antibody conjugated to Alexa594. The left panel shows staining of the nuclei with DAPI. A merge of the two stainings is shown on the right

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Last update: January 6, 2017