diagenode
A Hologic Company

TECHNICAL DATASHEET

H4K20me3 monoclonal antibody

Cat. No. C15100148
Type: Monoclonal **MeDIP-grade**
Source: Mouse
Lot #: DA-0022
Size: 100 µl
Concentration: Not determinedl

Specificity: Human, zebrafish: positive
Other species: not tested
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.

Description: Monoclonal antibody raised in mouse against histone H4 containing the trimethylated lysine 20 (H4K20me3), using a KLH-conjugated synthetic peptide.

Applications

	Suggested dilution	Results
ChIP*	3 µl/ChIP	Fig 1
Western blotting	1:50	Fig 2
Immunofluorescence	1:200	Fig 3

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

Results

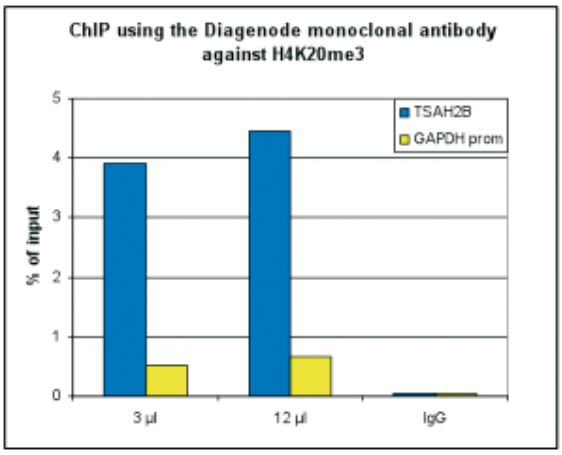

Figure 1. ChIP results obtained with the Diagenode monoclonal antibody directed against H4K20me3

ChIP assays were performed using human HeLa cells, the Diagenode monclonal antibody against H4K20me3 (Cat. No. C15100148) and optimized PCR primer sets for qPCR. ChIP was performed with the "LowCell# ChIP" kit (Cat. No. C01010072), 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).

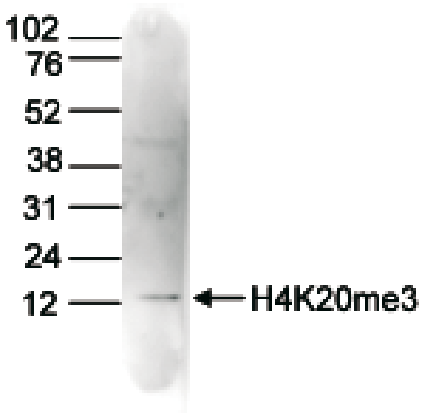

Figure 2. Western blot analysis using the Diagenode monoclonal antibody directed against H4K20me3

Histone extracts of HeLa cells (15 µg) were analysed by Western blot using the Diagenode monoclonal antibody against H4K20me3 (Cat. No. C15100148) diluted 1:50 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.

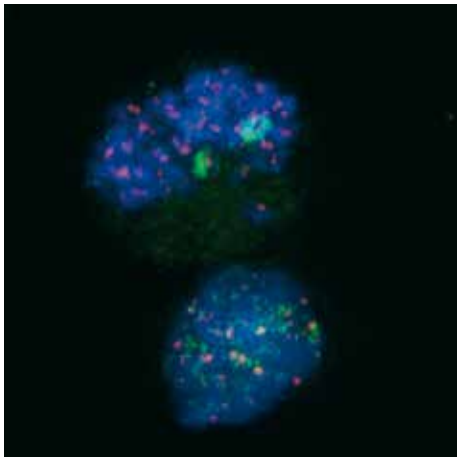

Figure 3. Immunofluorescence with the Diagenode monoclonal antibody directed against H4K20me3

HEK293T cells were stained with the Diagenode antibody against H4K20me3 (Cat. No. C15100148, green), with human autoantibodies against the centromeres (CREST, Cortex Biochem, pink) and with DAPI (blue). Cells were fixed with 4% paraformaldehyde and blocked with PBS containing 0.1% Tween, 2% BSA, and 5% normal goat serum. Cells were immunofluorescently labeled with the H4K20me3 antibody (diluted 1:200 in blocking solution) and the CREST antibody (diluted 1:1000 in blocking solution) by overnight incubation at 4°C. Subsequently cells were labeled with goat-anti-mouse antibody conjugated to Alexa488 and goat-anti-human antibody conjugated to Alexa633 (both diluted 1:200 in blocking solution and incubated for 1 hour at RT). Cells were mounted with Vectashield containing DAPI.

Immunofluorescence was performed by Dr. E. Baart, Erasmus University, Rotterdam, the Netherlands.

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