

H4K20me1 monoclonal antibody

Cat. No. C15200147

Type: Monoclonal ChIP grade/ChIP-seq grade

Isotype: IgG1

Source: Mouse

Lot #: 003

Size: 50 µg/18 µl

Concentration: 2.8 µg/µl

Specificity: Human, mouse: positive
Other species: not tested

Purity: Protein A purified monoclonal antibody in PBS containing 0.05% azide and 0.05% ProClin 300.

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 monomethylated lysine 20 (H4K20me1), using a KLH-conjugated synthetic peptide.

Applications

| | Suggested dilution | Results |
|------------------|--------------------|---------|
| ChIP* | 0,5 - 1 µg/ChIP | Fig 1,2 |
| Dot blotting | 1:20.000 | Fig 3 |
| Western blotting | 1:1.000 | Fig 4 |
| IF | 1:200 | Fig 5 |

* Please note that the optimal antibody amount per IP should be determined by the end-user. We recommend testing 0.5-5 µg per IP

Product 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

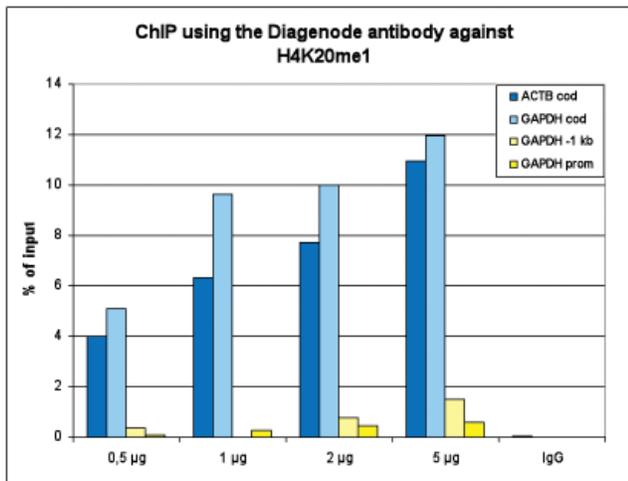


Figure 1. ChIP results obtained with the Diagenode monoclonal antibody directed against H4K20me1

ChIP assays were performed using human HeLa cells, the Diagenode monoclonal antibody against H4K20me1 [Cat. No. C15200147] and optimized PCR primer sets for qPCR. ChIP was performed with the “the iDeal ChIP-seq” kit [Cat. No. C01010051], using sheared chromatin from 1 million cells. A titration of the antibody consisting of 0.5, 1, 2 and 5 µg per ChIP experiment was analysed. IgG (1 µg/IP) was used as negative IP control. QPCR was performed with primers for the coding region of the active GAPDH and ACTB genes, used as positive controls, and for the GAPDH promoter and a region located 1 kb upstream of the GAPDH promoter, used as negative controls. Figure 1 shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).

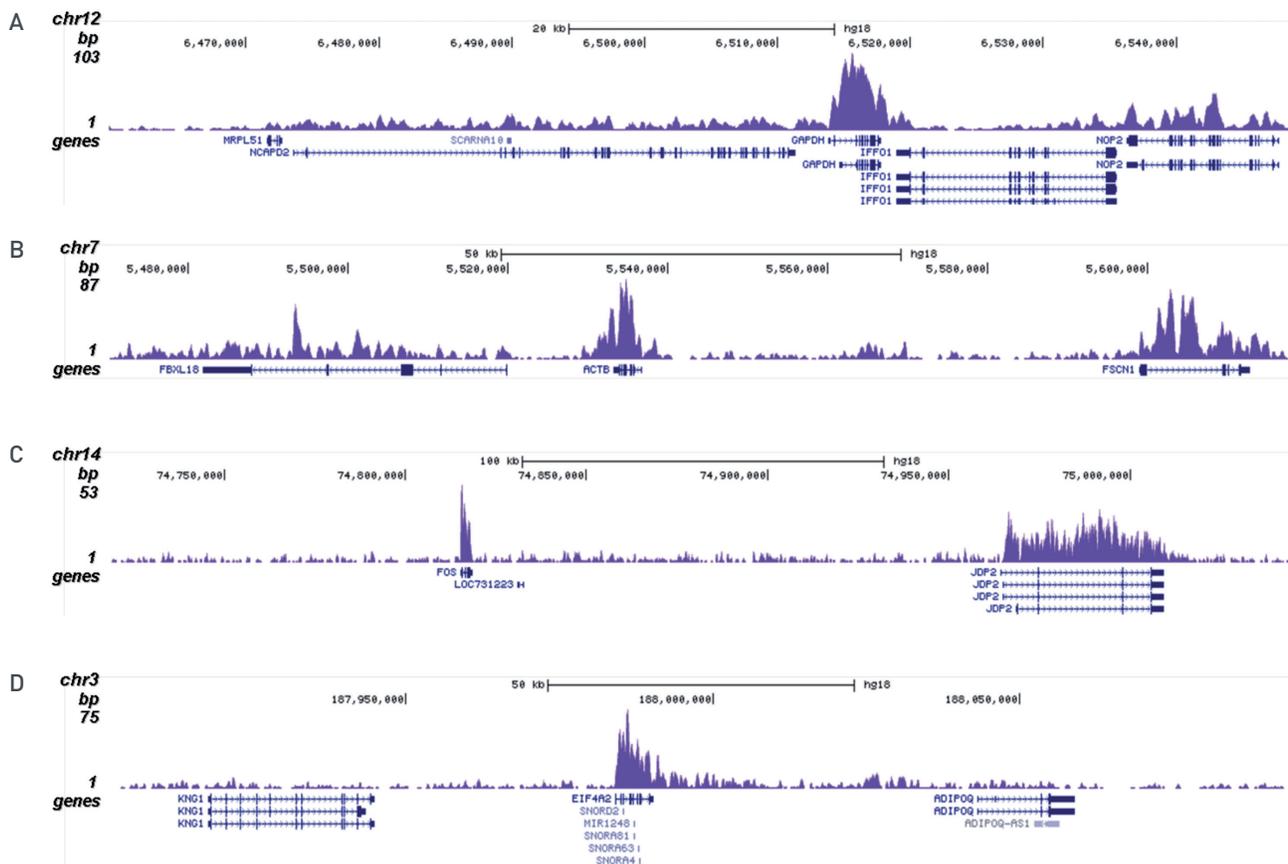


Figure 2. ChIP-seq results obtained with the Diagenode monoclonal antibody directed against H4K20me1

ChIP was performed as described above using 0.5 µg of the Diagenode antibody against H4K20me1 [Cat. No. C15200147]. The IP'd DNA was subsequently analysed on an Illumina HiSeq. Library preparation, cluster generation and sequencing were performed according to the manufacturer's instructions. The 51 bp tags were aligned to the human genome using the BWA algorithm. Figure 2 shows the peak distribution in genomic regions from chromosomes 12, 7, 14 and 3 surrounding the GAPDH, ACTB, FOS and EIF4A2 positive control genes (figure 2A, B, C and D, respectively).

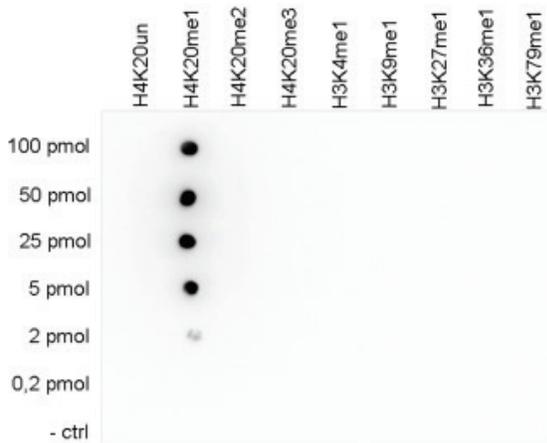


Figure 3. Cross reactivity tests using the Diagenode monoclonal antibody directed against H4K20me1

To check the specificity of the Diagenode monoclonal antibody against H4K20me1 [Cat. No C15200147] a Dot Blot was performed with peptides containing different modifications of histone H3 and H4 or the unmodified H4K20 sequence. 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 1:20,000. Figure 3 shows a high specificity of the antibody for the modification of interest.

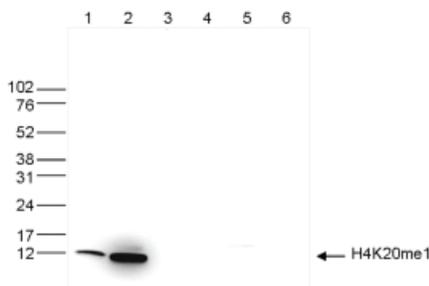


Figure 4. Western blot analysis using the Diagenode monoclonal antibody directed against H4K20me1

Western blot was performed on whole cell (25 µg, lane 1) and histone extracts (15 µg, lane 2) from HeLa cells, and on 1 µg of recombinant histone H2A, H2B, H3 and H4 (lane 3, 4, 5 and 6, respectively) using the Diagenode monoclonal antibody against H4K20me1 [Cat. No C15200147]. The antibody was diluted 1:1,000 in TBS-Tween containing 5% skimmed milk. The marker (in kDa) is shown on the left, the position of the protein is indicated on the right

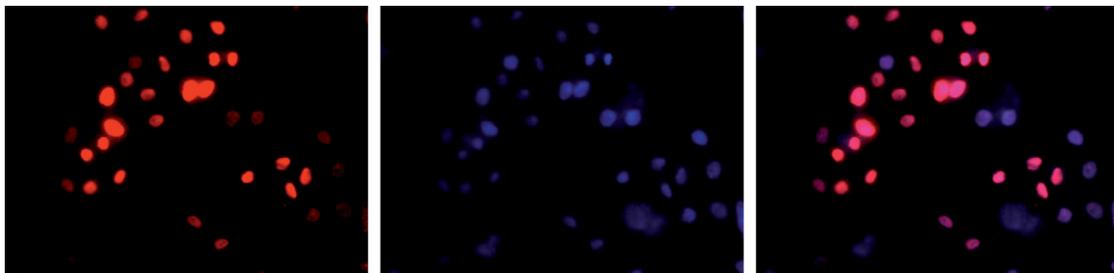


Figure 5. Immunofluorescence using the Diagenode monoclonal antibody directed against H4K20me1

HeLa cells were stained with the Diagenode antibody against H4K20me1 [Cat. No. C15200147] 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 H4K20me1 antibody (left) diluted 1:200 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.