diagenode
A Hologic Company

ChIP-seq grade

TECHNICAL DATASHEET

macroH2A.1/H2A.2 monoclonal antibody

Other name: H2AFY, H2A.Y, MH2A1, H2AF12M/ H2AFY2, MH2A2

Cat. No. C15210003
Type: Monoclonal **ChIP-grade / ChIP-seq grade**
Source: Rabbit
Lot #: 002
Size: 100 µg/100 µl
Concentration: 1 µg/µl

Specificity: Human: positive
Other species: not tested
Purity: Protein A purified monoclonal antibody in PBS containing 50% glycerol, 1% BSA and 0.09% 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 rabbit against histone macroH2A.1, using a KLH-conjugated synthetic peptide from the C-terminus of the protein. The antibody also recognizes macroH2A.2.

Applications

	Suggested dilution	Results
ChIP*	0.5 - 1 µg/ChIP	Fig 1, 2
Western blotting	1:2,000	Fig 3
Immunofluorescence	1:500	Fig 4

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

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. macroH2A.1 is an histone variant which replaces H2A in some nucleosomes and represses transcription. macroH2A.1 and macroH2A.2 are histone variants which replace H2A in some nucleosomes and repress transcription

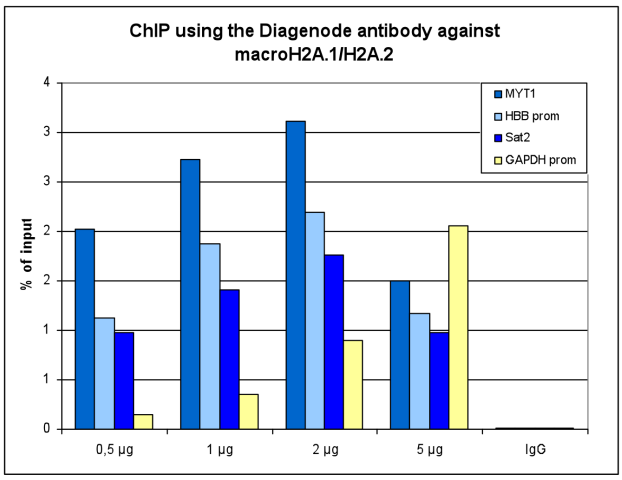

Figure 1. ChIP results obtained with the Diagenode monoclonal antibody directed against macroH2A.1/H2A.2

ChIP assays were performed using HeLa cells, the Diagenode antibody against macroH2A.1/H2A.2 (Cat. No. C15210003) and optimized PCR primer sets for qPCR. ChIP was performed with the "iDeal ChIP-seq" kit (Cat. No. C01010051), using sheared chromatin from 1 million cells. A titration consisting of 0.5, 1, 2 and 5 µg of antibody per ChIP experiment was analyzed. IgG (1 µg/IP) was used as a negative IP control. Quantitative PCR was performed with optimized primers for the MYT1 and HBB genes and for the Sat2 satellite repeat, used as positive controls, and for the GAPDH promoter, used as negative control.

Figure 1 shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).

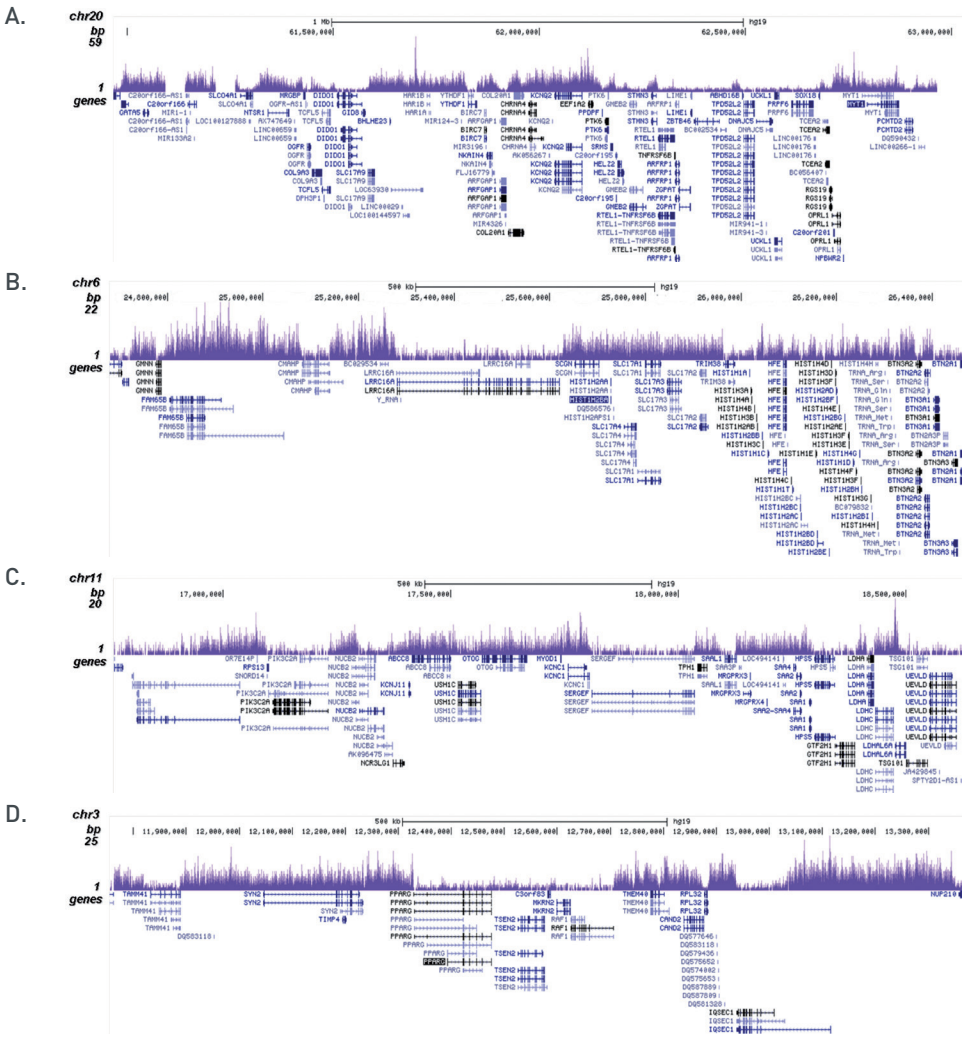

Figure 2. ChIP-seq results obtained with the Diagenode monoclonal antibody directed against macroH2A.1/H2A.2

ChIP was performed on sheared chromatin from 1 million HeLa cells using 0.5 µg of the Diagenode antibody against macroH2A.1/H2A.2 (Cat. No. C15210003) as described above. 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 50 bp tags were aligned to the human genome using the BWA algorithm. Figure 2 shows the enrichment in 4 genomic regions of chromosome 20 (including the MYT1 positive control gene), 6, 11 and 3, respectively.

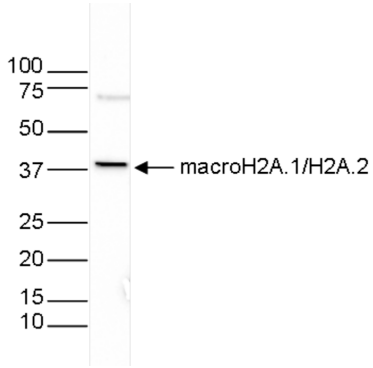

Figure 3. Western blot analysis using the Diagenode monoclonal antibody directed against macroH2A.1/H2A.2

Histone extracts from HeLa cells were analysed by Western blot using the Diagenode monoclonal antibody against macroH2A.1/H2A.2 (Cat. No. C15210003) diluted 1:2,000 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.

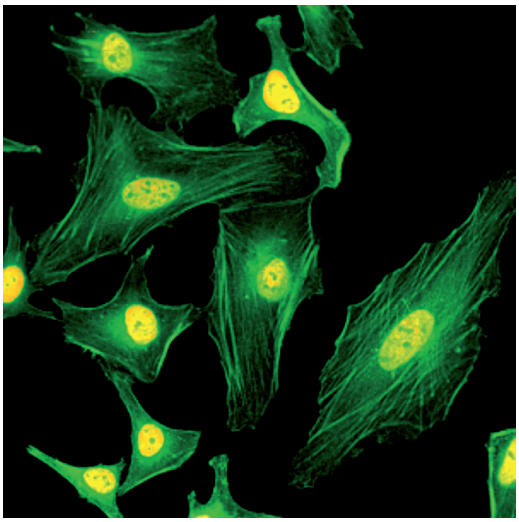

Figure 4. Immunofluorescence using the Diagenode monoclonal antibody directed against macroH2A.1/H2A.2

HeLa cells were stained with the Diagenode antibody against macroH2A.1/H2A.2 (Cat. No. C15210003, red) diluted 1:500. Actin filaments were stained with fluorescein phalladoin (green).

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