diagenode
Innovating Epigenetic Solutions

H3K9un monoclonal antibody

Cat. No. C15200187 (MAb-187-050)
Type: Monoclonal **ChIP grade**
Isotype: IgG1
Source: Mouse
Lot #: 001
Size: 50 µg/ 25 µl
Concentration: 2 µg/µl

Specificity: Human: 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 a region of histone H3 containing the unmodified lysine 9 (H3K9un), using a KLH-conjugated synthetic peptide.

Applications

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

*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 present in the 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

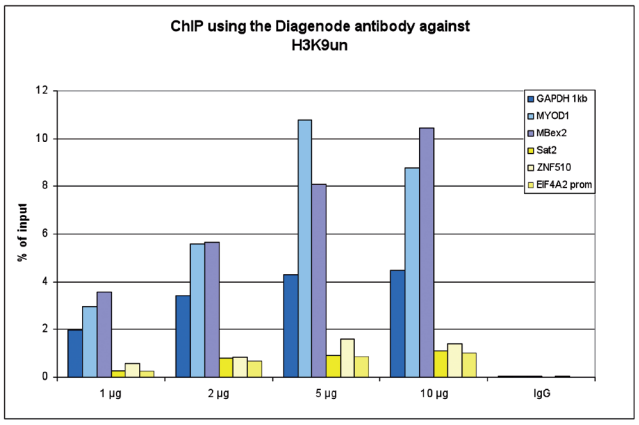

Figure 1. ChIP results obtained with the Diagenode monoclonal antibody directed against H3K9un

ChIP assays were performed using human HeLa cells, the Diagenode monclonal antibody against H3K9un (Cat. No. MAb-187-050) and optimized PCR primer sets for qPCR. ChIP was performed with the "Auto Histone ChIP-seq" kit (Cat. No. AB-Auto02-A100) on sheared chromatin from 1 million cells. using the SX-8G IP-Star automated system. A titration of the antibody consisting of 1, 2, 5, and 10 µg per ChIP experiment was analysed. IgG (2 µg/IP) was used as negative IP control. QPCR was performed with primers for the coding regions of the MYOD1 and MB genes and for a region 1 kb upstream of the GAPDH promoter, used as positive controls, and for the ZNF510 coding region, the EIF4A2 promoter and the Sat2 satellite repeat region, 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).

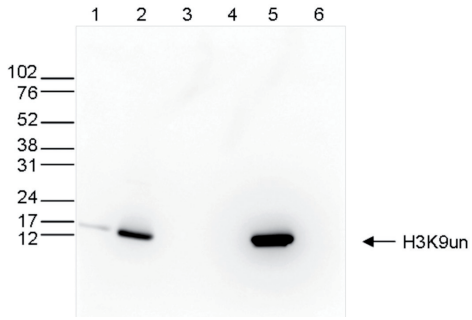

Figure 2. Western blot analysis using the Diagenode monoclonal antibody directed against H3K9un

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 antibody against H3K9un (Cat. No. MAb-187-050). The antibody was 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.

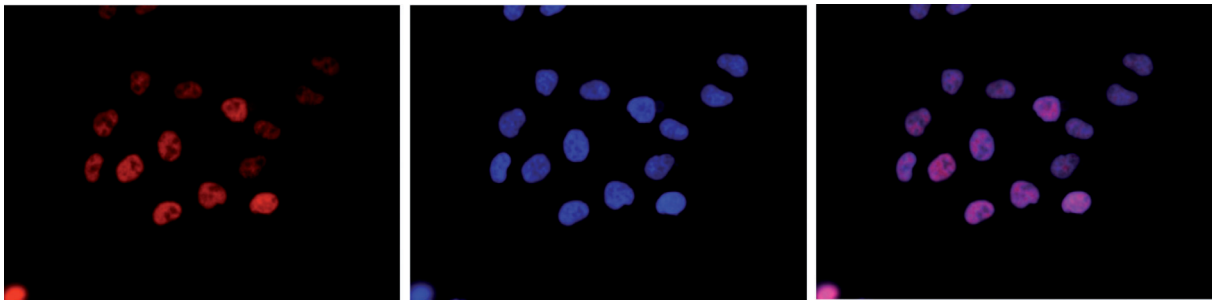

Figure 2. Immunofluorescence using the Diagenode monoclonal antibody directed against H3K9un

HeLa cells were stained with the Diagenode antibody against H3K9un (cat. No. MAb-187-050) 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 H3K9un 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.

Diagenode sa. BELGIUM | EUROPE
LIEGE SCIENCE PARK
Rue Bois Saint-Jean, 3
4102 Seraing (Ougrée) - Belgium
Tel: +32 4 364 20 50
Fax: +32 4 364 20 51
orders@diagenode.com
info@diagenode.com

Diagenode Inc. USA | NORTH AMERICA
400 Morris Avenue, Suite 101
Denville, NJ 07834 - USA
Tel: +1 862 209-4680
Fax: +1 862 209-4681
orders.na@diagenode.com
info.na@diagenode.com

Last update: November 9, 2012