diagenode
A Hologic Company

TECHNICAL DATASHEET

H3K36me3 monoclonal antibody

Cat. No. C15200183
Type: Monoclonal **ChIP grade**
IgG isotype: IgG1
Source: Mouse
Lot #: 001-12
Size: 50 µg/ 50 µl
Concentration: 1 µg/µl

Specificity: Human, rat: 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 H3 trimethylated at lysine 36 (H3K36me3), using a KLH-conjugated synthetic peptide.

Applications

	Suggested dilution	Results
ChIP*	0.5 - 1 µg per IP	Fig 1
ELISA	1:3,000	Fig 2
Dot blotting	1:10,000	
Western blotting	1:1,000 - 1:2,000	
IF	1:500	Fig 3

* Please note that the optimal antibody amount per ChIP should be determined by the end-user. We recommend testing 1-5 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.

Results

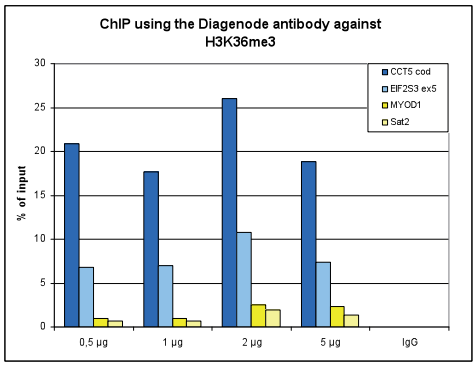

Figure 1. ChIP results obtained with the Diagenode antibody directed against H3K36me3

ChIP assays were performed using human HeLa cells, the Diagenode monoclonal antibody against H3K36me3 (cat. No. C15200183) and optimized PCR primer pairs for qPCR. ChIP was performed with the "Auto Histone ChIP-seq" kit (cat. No. C01010020), 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 primers specific for the coding regions of the active EIF2S3 and CCT5 genes, used as positive controls, and for the inactive MYOD1 gene and the Sat2 satellite repeat, 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).

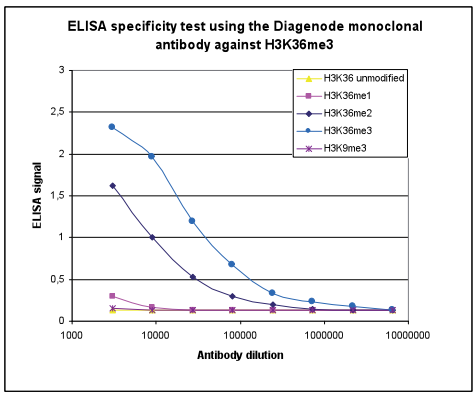

Figure 2. Cross reactivity of the Diagenode monoclonal antibody directed against H3K36me3

To test the specificity an ELISA was performed using a serial dilution of the Diagenode monoclonal antibody against H3K36me3 (cat. No. C15200183). The wells were coated with peptides containing the unmodified H3K36 region as well as the mono-, di- and trimethylated H3K36 and the trimethylated H3K9. Figure 2 shows a high specificity of the antibody for the peptide containing the modification of interest.

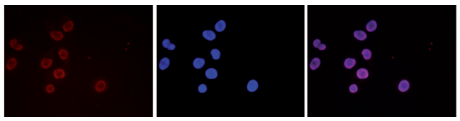

Figure 3. Immunofluorescence using the Diagenode monoclonal antibody directed against H3K36me3

HeLa cells were stained with the Diagenode antibody against H3K36me3 (cat. No. C15200183) and with DAPI. Cells were fixed with methanol and blocked with PBS/TX-100 containing 5% normal goat serum and 1% BSA. The cells were immunofluorescently labelled with the H3K36me3 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

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