diagenode
A Hologic Company

TECHNICAL DATASHEET

H3K4me2 polyclonal antibody

Cat. No. C15310035
Type: Polyclonal **ChIP-grade**
Source: Rabbit
Lot #: A391-001
Size: 100 µl
Concentration: Not determined

Specificity: Human, mouse: positive
Other species: not tested.
Purity: Whole antiserum from rabbit 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: Polyclonal antibody raised in rabbit against histone H3 containing the dimethylated lysine 4 (H3K4me2), using a KLH-conjugated synthetic peptide.

Applications

	Suggested dilution	References
ChIP *	10 µl/ChIP	Fig 1, 2
ELISA	1:300	Fig 3
Dot blotting	1:20,000	Fig 4
Western blotting	1:750	Fig 5

* 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. Methylation of histone H3K4 is associated with activation of gene transcription.

Results

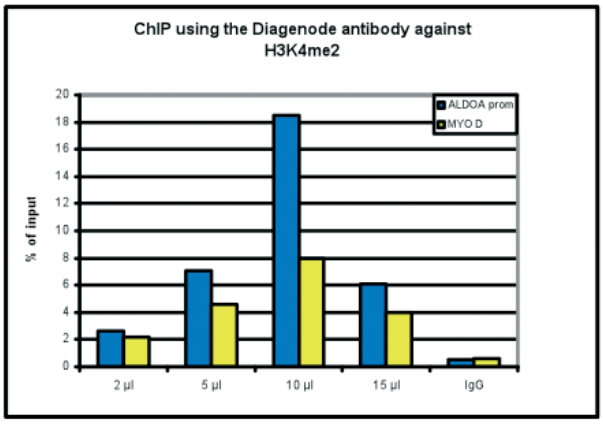

Figure 1. ChIP results obtained with the Diagenode antibody directed against H3K4me2

ChIP assays were performed using human osteosarcoma (U2OS) cells, the Diagenode antibody against H3K4me2 (Cat. No. C15310035) and optimized PCR primer sets for qPCR. Chromatin was sheared with the Diagenode "Shearing ChIP" kit (Cat. No. C01020020). ChIP was performed with the "OneDay ChIP" kit (Cat. No. C01010080), using sheared chromatin from 1.6 million cells. A titration of the antibody consisting of 2, 5, 10 or 15 µl per ChIP experiment was analysed. IgG (5 µg/IP) was used as negative IP control. Quantitative PCR was performed with primers for the promoter of the ALDOA gene and for the coding region of the myogenic differentiation gene (MYOD), a gene that is inactive at normal conditions. Figure 1 shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis). These results are in accordance with the observation that dimethylation of K4 at histone H3 is more present at active genes than at silent genes.

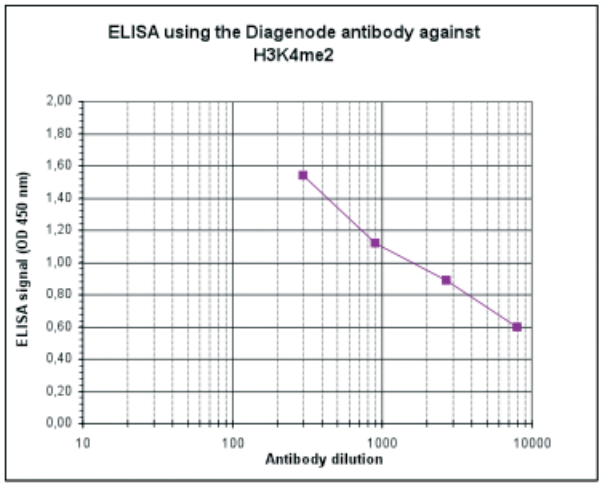

Figure 2. Determination of the titer

To determine the titer, an ELISA was performed using a serial dilution of the Diagenode antibody directed against H3K4me2 (Cat. No. C15310035). The antigen used was a peptide containing the histone modification of interest. By plotting the absorbance against the antibody dilution (Figure 2), the titer of the antibody was estimated to be 1:2,600.

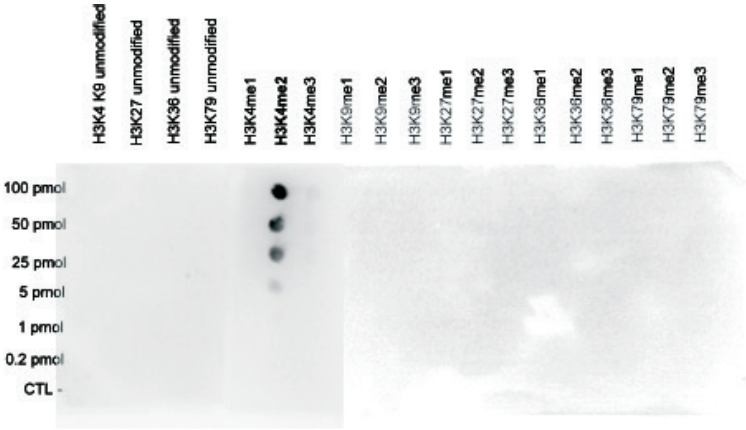

Figure 3. Cross reactivity test using the Diagenode antibody directed against H3K4me2

A Dot Blot analysis was performed to test the cross reactivity of the Diagenode antibody against H3K4me2 (Cat. No. C15310035) with peptides containing other modifications or unmodified sequences of histone H3. Other histone modifications include mono- and trimethylation of the same lysine and mono-, di- and trimethylation of lysine 9, 27 and 36 and 79. One hundred to 0.2 pmol of the peptides 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.

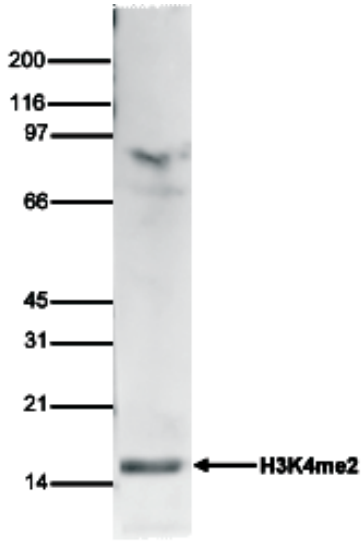

Figure 4. Western blot analysis using the Diagenode antibody directed against H3K4me2

Histone extracts of HeLa cells (15 µg) were analysed by Western blot using the Diagenode antibody against H3K4me2 (Cat. No. C15310035) diluted 1:750 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.

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: September 18, 2015