diagenode
A Hologic Company

TECHNICAL DATASHEET

PRODUCT NAME H3R17me2(asym) polyclonal antibody		
Cat. No. C15310092 (CS-092-100)	**Type:** Polyclonal **ChIP-grade**	**Size:** 100 µl
Lot #: A81-001	**Source:** Rabbit	**Concentration:** not determined

Product description: Polyclonal antibody raised in rabbit against histone H3 containing the asymmetrically dimethylated arginine 17 (H3R17me2(asym)), using a KLH-conjugated synthetic peptide.

Specificity: Human: positive
Other species: not tested

Applications	Suggested dilution	References
ChIP*	10 - 15 µl/ChIP	Fig 1
ELISA	1:1,000 – 1:3,000	Fig 2
Dot blotting	1:20,000	Fig 3
Western blotting	1:250	Fig 4

*Please note that of the optimal antibody amount per IP should be determined by the end-user. We recommend testing 1-15 µl per IP.

Purity: Whole antiserum from rabbit containing 0.05% azide.

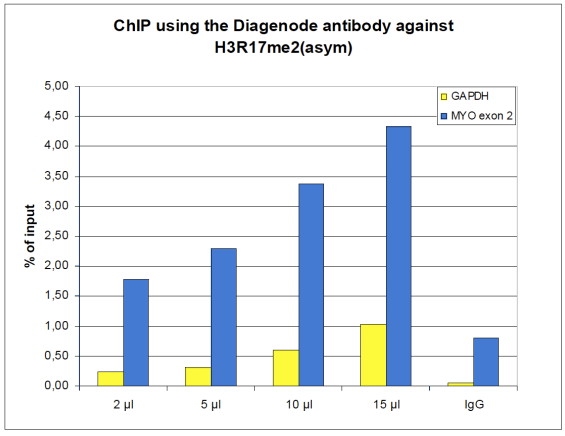
Storage: Store at -20°C; for long storage, store at -80°C. Avoid multiple freeze-thaw cycles.

Precautions: This product is for research use only. Not for use in diagnostic or therapeutic procedures.

Last data sheet update: April 22, 2011

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.

A



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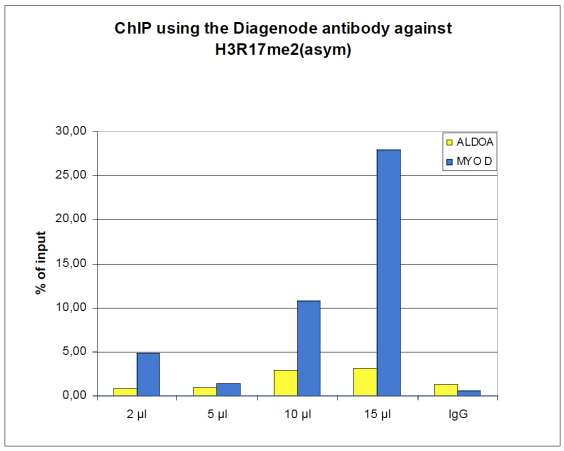

Figure 1

ChIP results obtained with the Diagenode antibody directed against H3R17me2(asym)

ChIP assays were performed using human osteosarcoma (U2OS) cells, the Diagenode antibody against H3R17me2(asym) (cat. No. CS-092-100) and optimized PCR primer sets for qPCR. Chromatin was sheared with the Diagenode "Shearing ChIP" kit (cat. No. kch-redmod-100). ChIP was performed with the "OneDay ChIP" kit (cat. No. kch-oneDIP-060), using sheared chromatin from 1.6 million cells per ChIP reaction. A titration of the antibody consisting of 2, 5, 10 and 15 µl per ChIP experiment was analysed. IgG (5 µg/IP) was used as negative IP 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 1A: QPCR performed with primers for the GAPDH promoter (cat. No. pp-1001-050) and for exon 2 of the myoglobin gene (cat. No. pp-1006-050).

Figure 1B: QPCR performed with primers for the promoter of the active ALDOA gene and for the coding region of the inactive MYOD gene..

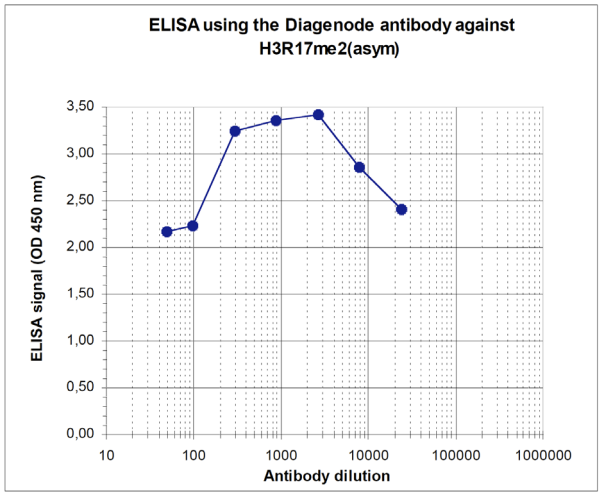

Figure 2

Determination of the titer

To determine the titer, an ELISA was performed using a serial dilution of the Diagenode antibody directed against H3R17me2(asym) (cat. No. CS-092-100). 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 1:40,000.

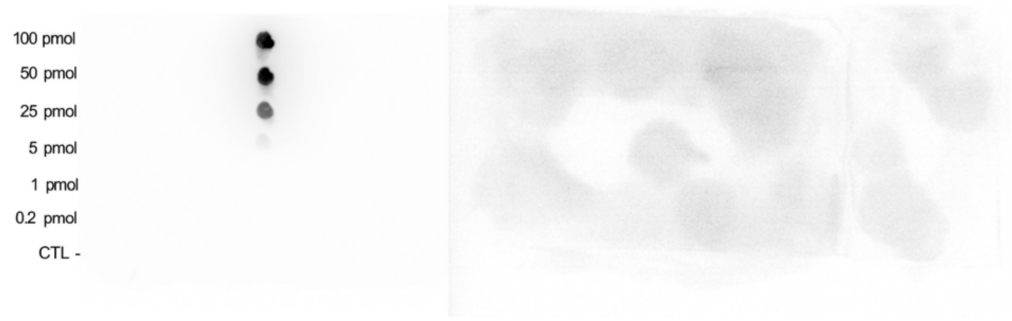

Figure 3

Cross reactivity test using the Diagenode antibody directed against H3R17me2(asym)

A Dot Blot analysis was performed to test the cross reactivity of the Diagenode antibody against H3R17me2(asym) (cat. No. CS-092-100) with peptides containing other modifications of histone H3 and H4 and unmodified sequences from histone H3. One hundred to 0.2 pmol of the 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.

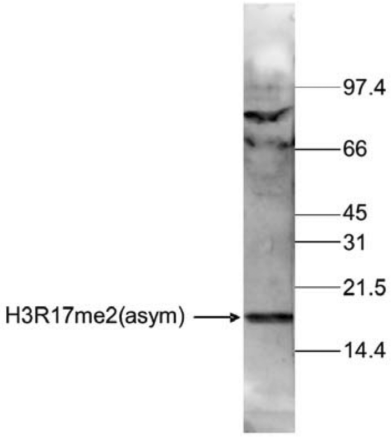

Figure 4

Western blot analysis using the Diagenode antibody directed against H3R17me2(asym)

Histone extracts of HeLa cells (15 µg) were analysed by Western blot using the Diagenode antibody against H3R17me2(asym) (cat. No. CS-092-100) diluted 1:250 in TBS-Tween containing 5% skimmed milk. The position of the protein of interest is indicated on the left; the marker (in kDa) is shown on the right.