

## H3R17me2(asym)K18ac polyclonal antibody

**Cat. No.** C15410171

**Type:** Polyclonal  
**ChIP-grade / ChIP-seq-grade**

**Source:** Rabbit

**Lot #:** A1922-001P

**Size:** 50 µg/ 53 µl

**Concentration:** 0.94 µg/µl

**Specificity:** Human, mouse, wide range expected

**Purity:** Affinity purified polyclonal 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:** Polyclonal antibody raised in rabbit against the region of histone H3 containing the asymmetrically dimethylated R17 and the acetylated lysine 18 (H3R17me2(asym)K18ac), using a KLH-conjugated synthetic peptide.

### Applications

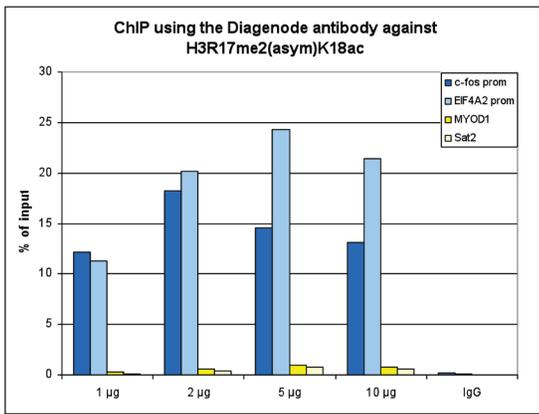
	Suggested dilution	Results
ChIP*	1 µg per IP	Fig 1, 2
ELISA	1:1,000	Fig 3
Dot blotting	1:20,000	Fig 4
Western blotting	1:1,000	Fig 5
IF	1:500	Fig 6

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

### Target description

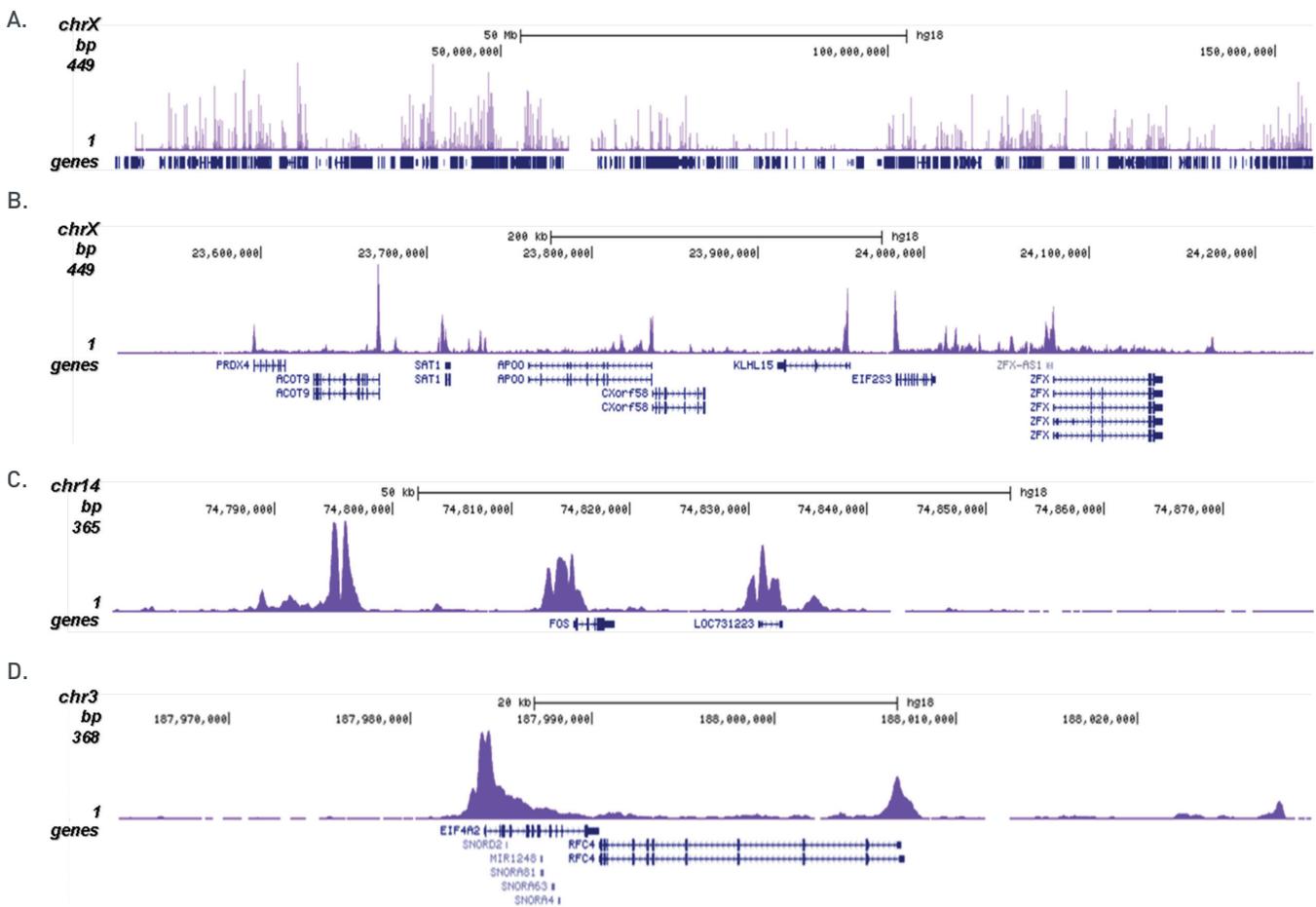
Histones are the main constituents of the protein part of chromosomes of eukaryotic cells. They are rich in the amino acids arginine and lysine and have been greatly conserved during evolution. Histones pack the DNA into tight masses of chromatin. Two core histones of each class H2A, H2B, H3 and H4 assemble 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.

## Results



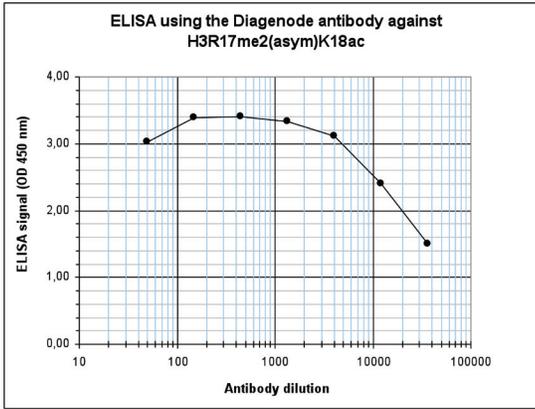
**Figure 1.** ChIP results obtained with the Diagenode antibody directed against H3R17me2(asym)K18ac

ChIP assays were performed using human HeLa cells, the Diagenode antibody against H3R17me2(asym)K18ac (cat. No. C15410171) and optimized PCR primer pairs for qPCR. ChIP was performed with the “iDeal ChIP-seq” kit (cat. No. AB-001-0024), using sheared chromatin from 1,000,000 cells. A titration consisting of 1, 2, 5 and 10 µg of antibody per ChIP experiment was analyzed. IgG (2 µg/IP) was used as a negative IP control. Quantitative PCR was performed with primers for the promoters of the active EIF4A2 and c-fos 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].



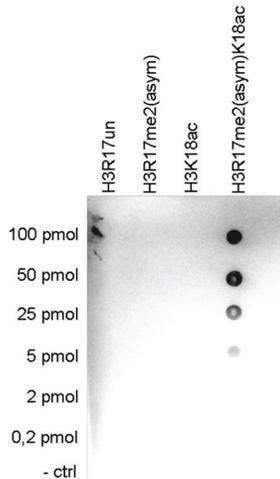
**Figure 2.** ChIP-seq results obtained with the Diagenode antibody directed against H3R17me2(asym)K18ac

ChIP was performed as described above using 1 µg of the Diagenode antibody against H3R17me2(asym)K18ac (cat. No. C15410171). The IP'd DNA was subsequently analysed on an Illumina Genome Analyzer. Library preparation, cluster generation and sequencing were performed according to the manufacturer's instructions. The 36 bp tags were aligned to the human genome using the ELAND algorithm. Figure 2 shows the peak distribution along the complete human X-chromosome and a zoomin to a 500 kb region (figure 2A and B), and in two regions on chromosome 14 and 3 surrounding the c-fos and EIF4A2 positive control genes (figure 2C and D, respectively).



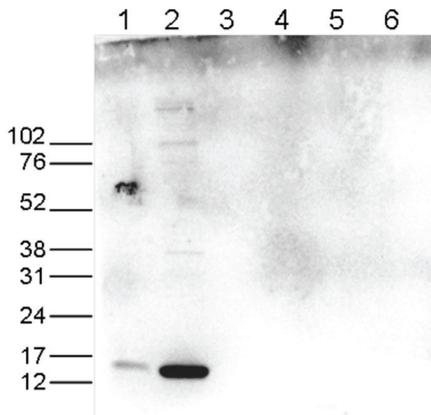
**Figure 3. Determination of the antibody titer**

To determine the titer of the antibody, an ELISA was performed using a serial dilution of the Diagenode antibody against H3R17me2(asym)K18ac (cat. No. C15410171). The antigen used was a peptide containing the histone modification of interest. By plotting the absorbance against the antibody dilution (Figure 3), the titer of the antibody was estimated to be 1:28,800.



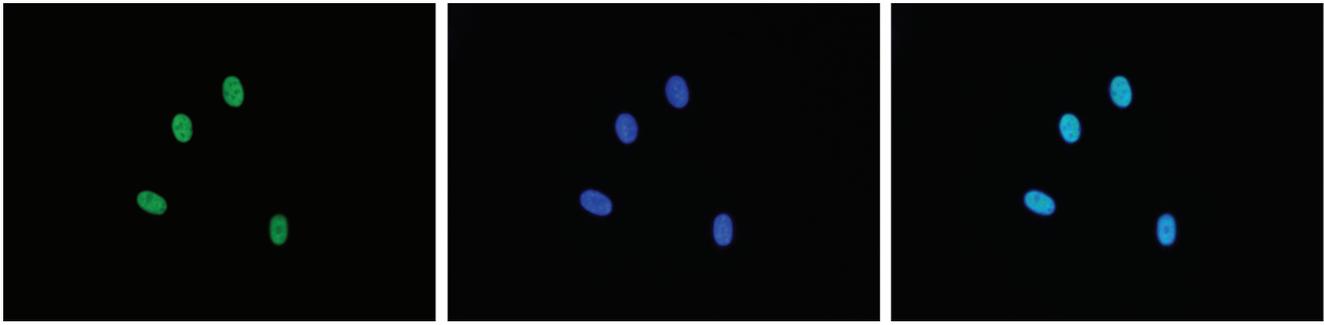
**Figure 4. Cross reactivity tests using the Diagenode antibody directed against H3R17me2(asym)K18ac**

To test the cross reactivity of the Diagenode antibody against H3R17me2(asym)K18ac (cat. No. C15410171), a Dot Blot analysis was performed with peptides containing other histone modifications and the unmodified H3R17K18. One hundred to 0.2 pmol of the respective peptides were spotted on a membrane. The antibody was used at a dilution of 1:20,000. Figure 4 shows a high specificity of the antibody for the modification of interest.



**Figure 5. Western blot analysis using the Diagenode antibody directed against H3R17me2(asym)K18ac**

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 H3R17me2(asym)K18ac (cat. No. C15410171). The antibody was diluted 1:1,000 in TBS-Tween containing 5% skimmed milk. The marker (in kDa) is shown on the left.



**Figure 6. Immunofluorescence using the Diagenode antibody directed against H3R17me2(asym)K18ac**

Mouse NIH3T3 cells were stained with the Diagenode antibody against H3R17me2(asym)K18ac (cat. No. C15410171) 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 labeled with the H3R17me2(asym)K18ac antibody (left) diluted 1:500 in blocking solution followed by an anti-rabbit antibody conjugated to Alexa488. 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:** February 6, 2013