diagenode
A Hologic Company

TECHNICAL DATASHEET

H4R3me2(sym) polyclonal antibody - Classic

Cat. No. C15410308
Type: Polyclonal
Source: Rabbit
Lot #: A2274P
Size: 50 µg/84 µl
Concentration: 0.6 µg/µl

Specificity: Human: positive / Other species: not tested
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 H4 containing the symmetrically dimethylated arginine 3 (H4R3me2(sym)), using a KLH-conjugated synthetic peptide. The antibody also recognizes H2AR3me2(sym).

Applications

	Suggested dilution/amount	Results
ELISA	1:500	Fig 1
Dot blotting	1:5,000	Fig 2
WB	1:1,000	Fig 3
IF	1:500	Fig 4

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, H2A, 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

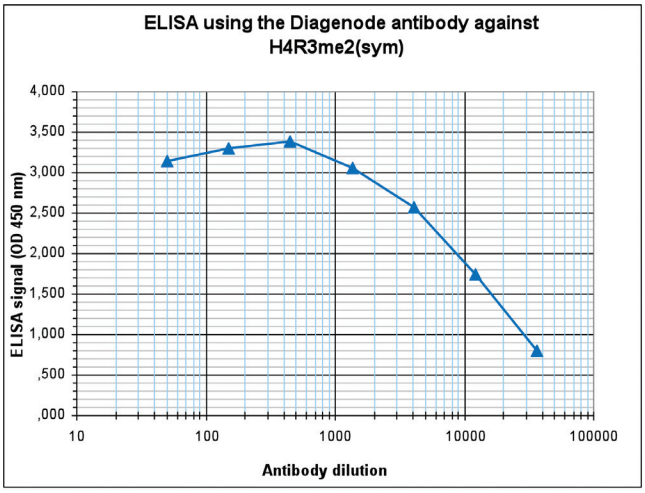
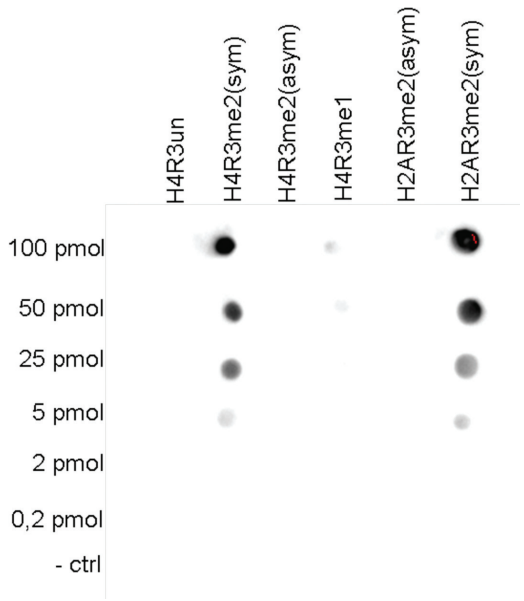


Figure 1. Determination of the antibody titer

To determine the titer of the antibody, an ELISA was performed using a serial dilution of the Diagenode antibody directed against H4R3me2(sym) (cat. No. 15410308) in antigen coated wells. The antigen used was a peptide containing the histone modification of interest. By plotting the absorbance against the antibody dilution (Figure 1), the titer of the antibody was estimated to be 1:11,750.

Figure 2. Cross reactivity tests using the Diagenode antibody directed against H4R3me2(sym)

To test the cross reactivity of the Diagenode antibody against H4R3me2(sym) (cat. No. 15410308), a Dot Blot analysis was performed with peptides containing other histone arginine methylations and the unmodified H4R3. One hundred to 0.2 pmol of the respective peptides were spotted on a membrane. The antibody was used at a dilution of 1:5,000. Figure 2 shows the antibody is specific for the H4R3 symmetric dimethylation and also recognizes the H2AR3 symmetric dimethylation.



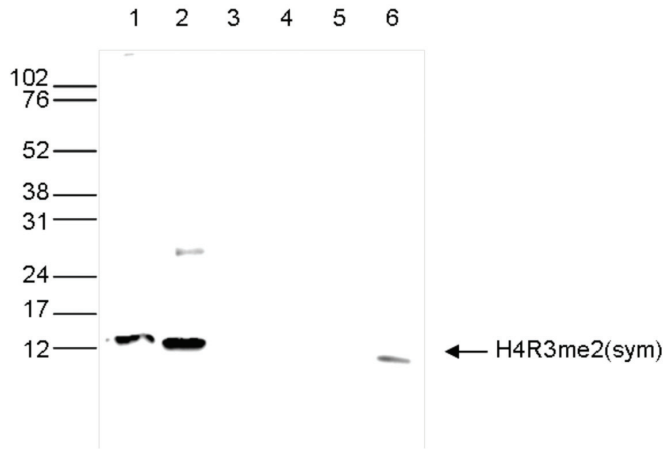


Figure 3. Western blot analysis using the Diagenode antibody directed against H4R3me2(sym)

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 H4R3me2(sym) (cat. No. C15410308). 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.

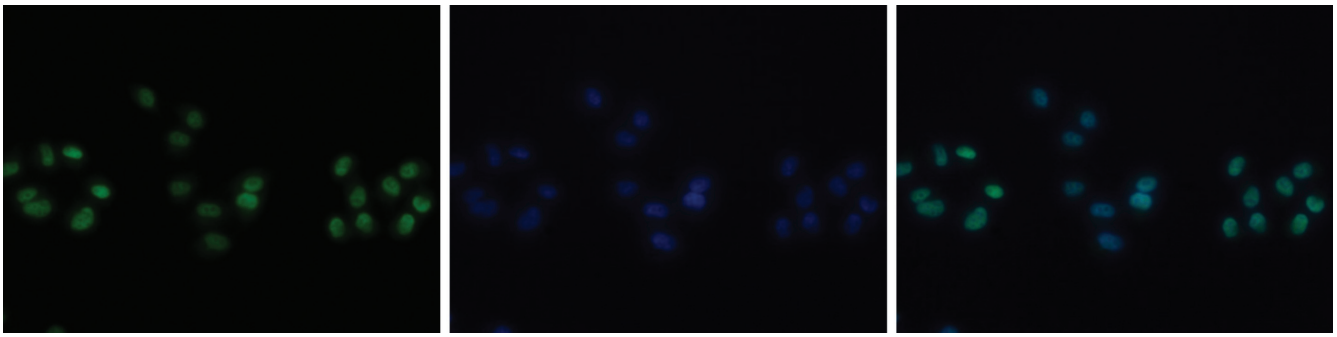


Figure 4. Immunofluorescence using the Diagenode antibody directed against H4R3me2(sym)

HeLa cells were stained with the Diagenode antibody against H4R3me2(sym) (cat. C15410308) 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 H4R3me2(sym) 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: October 27, 2014