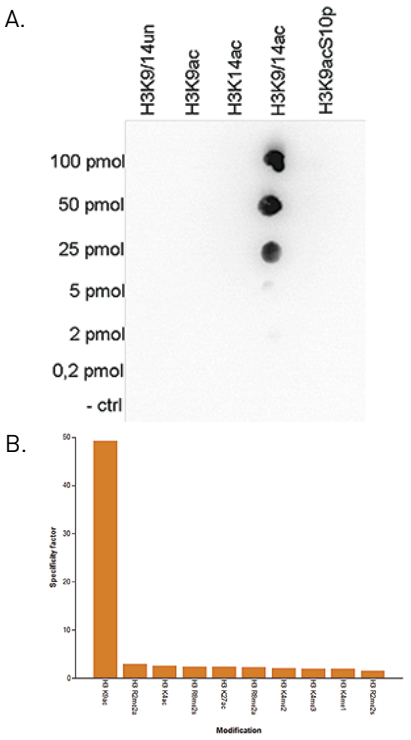

Figure 5. Cross reactivity tests using the Diagenode antibody directed against H3K9/14ac

Figure 5A To test the cross reactivity of the Diagenode antibody against H3K9/14ac (Cat. No. C15410200), a Dot Blot analysis was performed with peptides containing other histone modifications and the unmodified H3K9. 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 5A shows a high specificity of the antibody for the modification of interest. Figure 5B The specificity of the antibody was further demonstrated by peptide array analyses on an array containing 384 peptides with different combinations of modifications from histone H3, H4, H2A and H2B. The antibody was used at a dilution of 1:2,000. Figure 5B shows the specificity factor, calculated as the ratio of the average intensity of all spots containing the mark, divided by the average intensity of all spots not containing the mark.

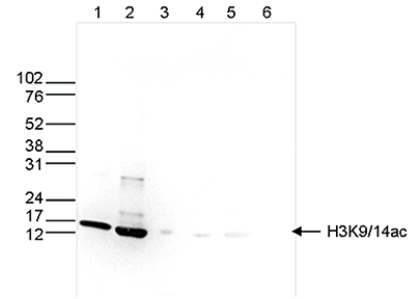

Figure 6. Western blot analysis using the Diagenode antibody directed against H3K9/14ac

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 H3K9/14ac (Cat. No. C15410200). The antibody was diluted 1:500 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.

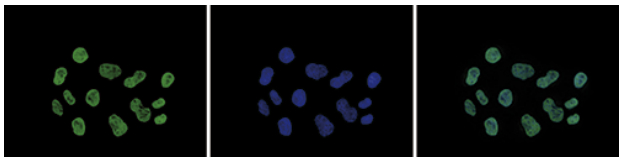

Figure 7. Immunofluorescence using the Diagenode antibody directed against H3K9/14ac

HeLa cells were stained with the Diagenode antibody against H3K9/14ac (Cat. No. C15410200) 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 H3K9/14ac antibody (left) diluted 1:200 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. BELIGUM | 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
info@diagenode.com | orders@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
info.na@diagenode.com | orders.na@diagenode.com