diagenode
A Hologic Company

TECHNICAL DATASHEET

H2A.XS139p polyclonal antibody

Other names: gamma H2A.X

Cat. No. C15410219
Type: Polyclonal
Source: Rabbit
Lot #: A2099P
Size: 50 µg / 19 µl
Concentration: 2.75 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 H2A.X containing the phosphorylated serine 139 (H2A.XS139p), using a KLH-conjugated synthetic peptide

Applications

	Suggested dilution	Results
ELISA	1:10,000	Fig 1
Dot blotting	1:10,000	Fig 2
Western blotting	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, 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. H2A.XS139p appears during apoptosis and is probably involved in DNA repair.

Results

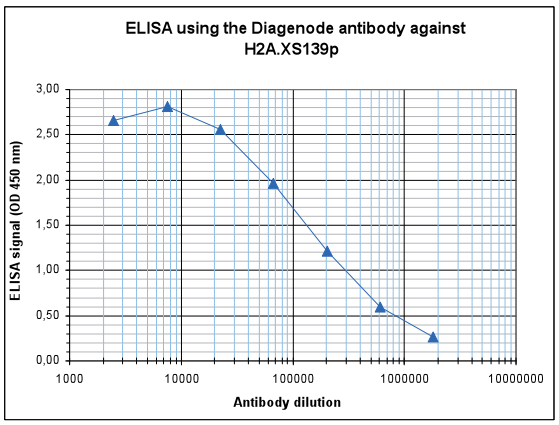


Figure 1. Determination of the antibody titer

To determine the titer of the antibody, an ELISA was performed using a serial dilution of the Diagenode antibody directed against H2A.XS139p (Cat. No. C15410219) 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 1:170,000.

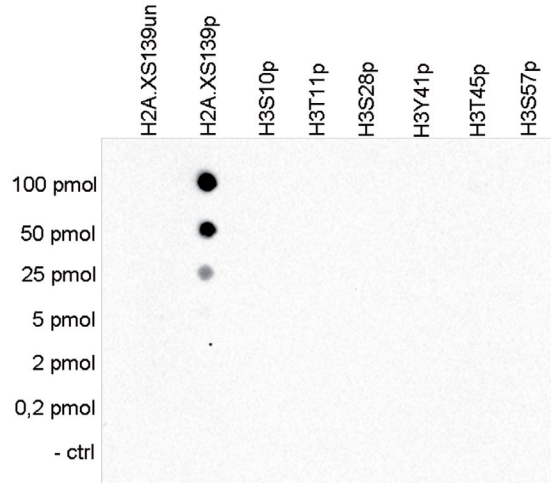


Figure 2. Cross reactivity tests using the Diagenode antibody directed against H2A.XS139p

To test the cross reactivity of the Diagenode antibody against H2A.XS139p (Cat. No. C15410219), a Dot Blot analysis was performed with peptides containing other histone phosphorylations and the unmodified H2A.X. One hundred to 0.2 pmol of the respective peptides were spotted on a membrane. The antibody was used at a dilution of 1:10,000. Figure 2 shows a high specificity of the antibody for the modification of interest.

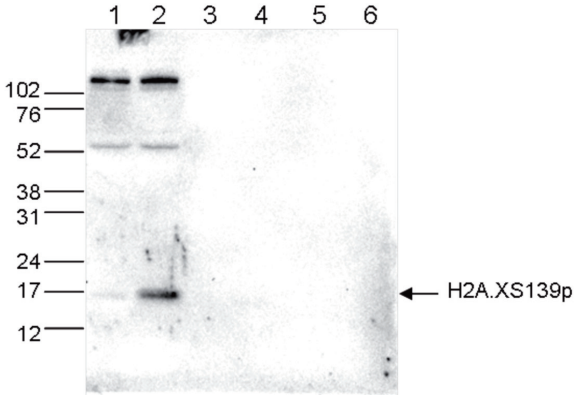
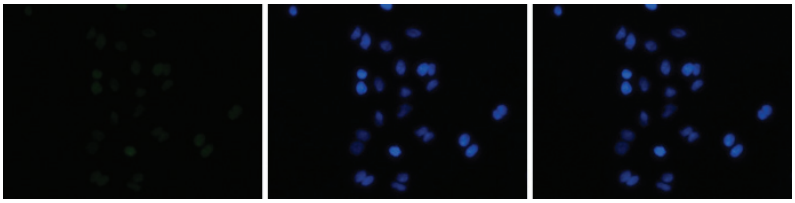


Figure 3. Western blot analysis using the Diagenode antibody directed against H2A.XS139p

Western blot was performed on histone extracts (15 µg) from untreated U2OS cells (lane 1) or from U2OS cells treated with camptothecin (lane 2), and on 1 µg of recombinant histone H2A, H2B, H3 and H4 (lane 3, 4, 5 and 6, respectively) using the Diagenode antibody against H2A.XS139p (Cat. No. C15410219). The antibody was diluted 1:1,000 in TBS-Tween containing 5% skimmed milk. The marker (in kDa) is shown on the left.

Results

A



B

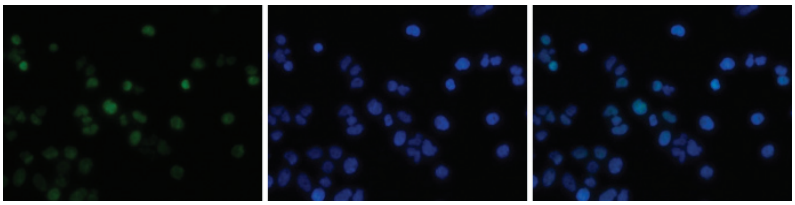


Figure 4. Immunofluorescence using the Diagenode antibody directed against H2A.XS139p

U2OS cells, either treated with camptothecin (figure 4B) or untreated (figure 4A), were stained with the Diagenode antibody against H2A.XS139p (cat. No. C15410219) 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 labelled with the H2A.XS139p 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.

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Last update: September 3, 2014