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H3T11 Phosph polyclonal antibody - Classic
Purified rabbit polyclonal Antibody
Catalog # ADN10286

Specification

H3T11 Phosph polyclonal antibody - Classic - Product Information

Application	**CHIP, E, DB, WB**
Primary Accession	Q93081
Reactivity	**Human**
Host	**Rabbit**
Clonality	**Polyclonal**

H3T11 Phosph polyclonal antibody - Classic - Additional Information

Target/Specificity
H3T11

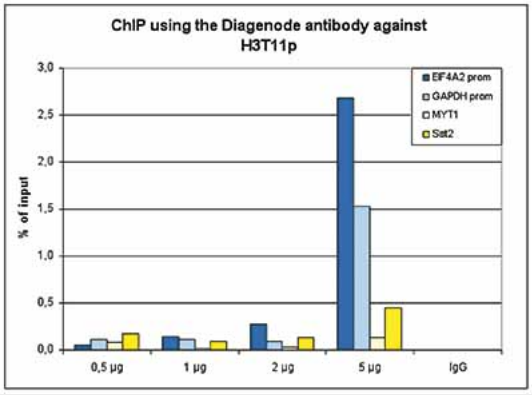
Precautions
H3T11 Phosph polyclonal antibody - Classic is for research use only and not for use in diagnostic or therapeutic procedures.

H3T11 Phosph polyclonal antibody - Classic - Protein Information

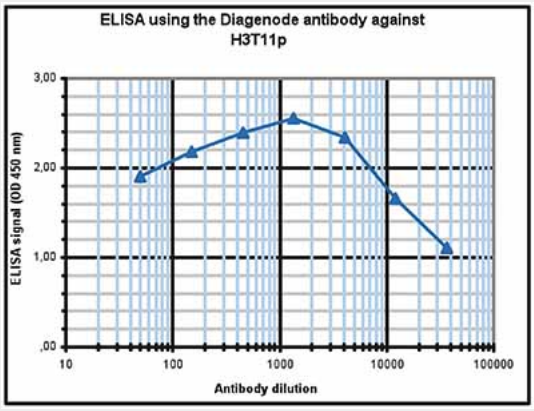
H3T11 Phosph polyclonal antibody - Classic - Protocols

Provided below are standard protocols that you may find useful for product applications.

- Western Blot
- Blocking Peptides
- Dot Blot
- Immunohistochemistry
- Immunofluorescence
- Immunoprecipitation
- Flow Cytomety
- Cell Culture



ChIP assays were performed using human HeLa cells, treated with colcemid, the Diagenode antibody against H3T11p (cat. No. ADN10286) and optimized PCR primer sets for qPCR. ChIP was performed with the “iDeal ChIP-seq” kit (cat. No. C01010055), using sheared chromatin from 1.5 million cells. A titration of the antibody consisting of 0.5, 1, 2 and 5 µg per ChIP experiment was analysed. IgG (2 µg/IP) was used as negative IP control. QPCR was performed with primers for the EIF4A2 and GAPDH promoters, used as positive controls, and for the coding region of the inactive MYT1 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).



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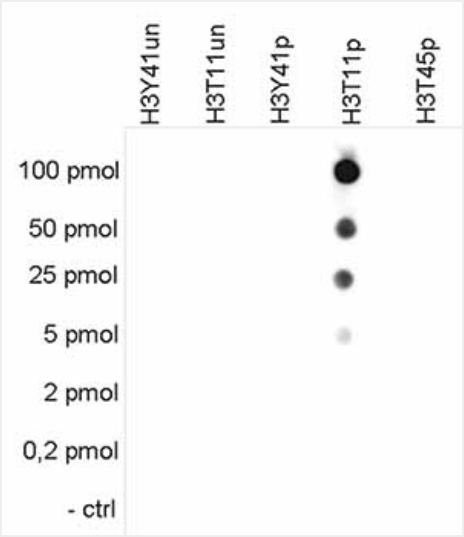
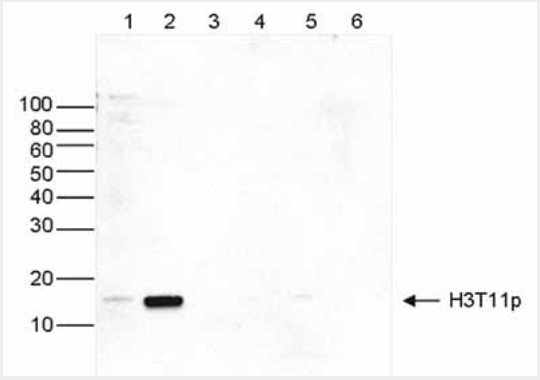
To determine the titer of the antibody, an ELISA was performed using a serial dilution of the Diagenode antibody directed against H3T11p (cat. No. ADN10286) in antigen coated wells. The antigen used was a peptide containing the histone modification of interest. By plotting the absorbance against the antibody dilution (Figure 2), the titer of the antibody was estimated to be 1:25,700.



To test the cross reactivity of the Diagenode antibody against H3T11p (cat. No. ADN10286), a Dot Blot analysis was performed with peptides containing other histone phosphorylations and the unmodified H3T11. 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 3 shows a high specificity of the antibody for the modification of interest.



Western blot was performed on whole cell extracts from untreated HeLa cells (25 µg, lane 1), on histone extracts from HeLa cells treated with colcemid (15 µg, lane 2), and on 1 µg of recombinant histone H2A, H2B, H3

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and H4 (lane 3, 4, 5 and 6, respectively) using the Diagenode antibody against H3T11p (cat. No. ADN10286). The antibody was diluted 1:1,000 in TBS-Tween containing 5% skimmed milk. The marker (in kDa) is shown on the left.