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H3pan polyclonal antibody - Classic

Purified rabbit polyclonal Antibody

Catalog # ADN10293

Specification

H3pan polyclonal antibody - Classic - Product Information

Application	**CHIP, E, WB, IF**
Primary Accession	O93081
Reactivity	**Human**
Host	**Rabbit**
Clonality	**Polyclonal**

H3pan polyclonal antibody - Classic - Additional Information

Target/Specificity
H3pan

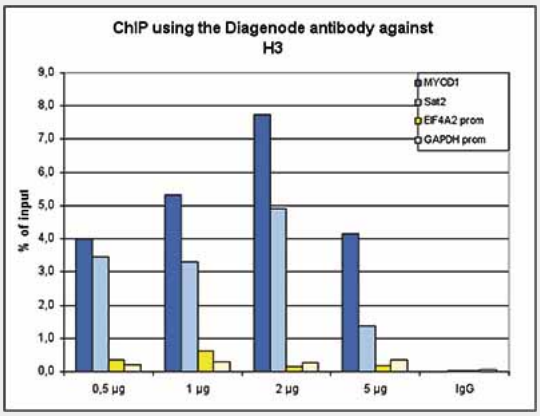
Precautions
H3pan polyclonal antibody - Classic is for research use only and not for use in diagnostic or therapeutic procedures.

H3pan polyclonal antibody - Classic - Protein Information

H3pan 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, the Diagenodeantibody against H3 (Cat. No. ADN10293) and optimized PCR primer pairs for qPCR. ChIP was performed with the “Auto Histone ChIP-seq” kit (Cat. No. C01010022), using sheared chromatin from 1 million cells on the IP-Star Compact automated system. A titration consisting of 0.5, 1, 2 and 5 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 specific for the promoters of the active GAPDH and EIF4A2 genes, and for the inactive MYOD1 gene and the Sat2 satellite repeat. 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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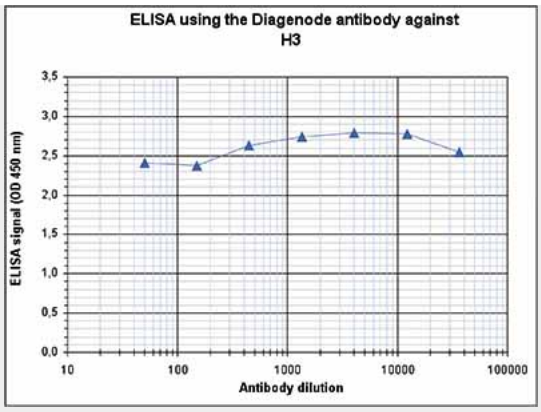
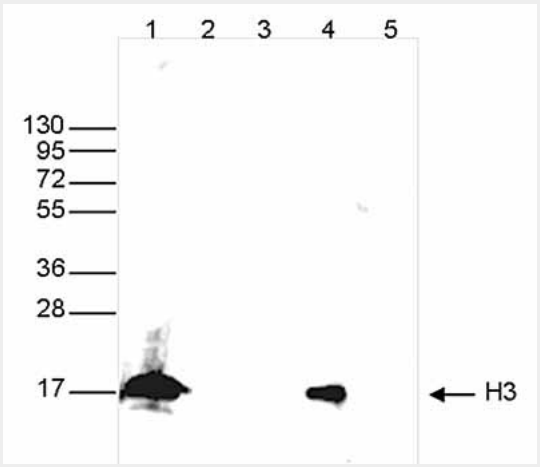


To determine the titer of the antibody, an ELISA was performed using a serial dilution of the Diagenode antibody directed against H3pan (Cat. No. ADN10293). The plates were coated with the peptides used for immunization. By plotting the absorbance against the antibody dilution (Figure 2), the titer of the antibody was estimated to be >1:1,000,000.



Western blot was performed on whole cell extracts from HeLa cells (25 µg, lane 1), and on 1 µg of recombinant histone H2A, H2B, H3 and H4 (lane 2, 3, 4 and 5, respectively) using the Diagenode antibody against H3 (Cat. No. ADN10293). 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.

HeLa cells were stained with the Diagenode

antibody against H3 (Cat. No. ADN10293) and with DAPI. Cells were fixed with 4% formaldehyde for 10’ and blocked with PBS/TX-100 containing 1% BSA. The cells were immunofluorescently labeled with the H3 antibody (middle) diluted 1:500 in blocking solution followed by an anti-rabbit antibody conjugated to Alexa488. The left panel shows staining of the nuclei with DAPI. A merge of the two stainings is shown on the right.