Diagenode sa
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Technical Data Sheet

Product name:
antibody directed against H3K9ac

(Histone H3 [acetylated lysine 9])

Catalog #: pAb-ACHAHS-044 (also pAb-004-044)	**Type:** Polyclonal	**Size:** 44 µg/ 20 µl
Lot #: DA-0010	**Source:** Rabbit	**Concentration:** 2.2 µg/µl

Description: This antibody has been raised against the region of the histone H3 containing the acetylated lysine 9 (or [K9ac]), using a KLH-conjugated synthetic peptide.

Specificity: Human: positive
Other species: not tested

Applications	Suggested dilution	References
ELISA	Tested	Fig 1
Dot blotting	Tested	
Western blotting	Not tested	
Gel Supershift	Not tested	
Immunofluorescence	1:200	Fig 3
Flow cytometry	Not tested	
Immunoprecipitation	Not tested	
ChIP	2 µg per IP	Fig 2

Format: In solution in PBS containing 0.01% azide and 0.1% BSA. The polyclonal antibody has been affinity purified.

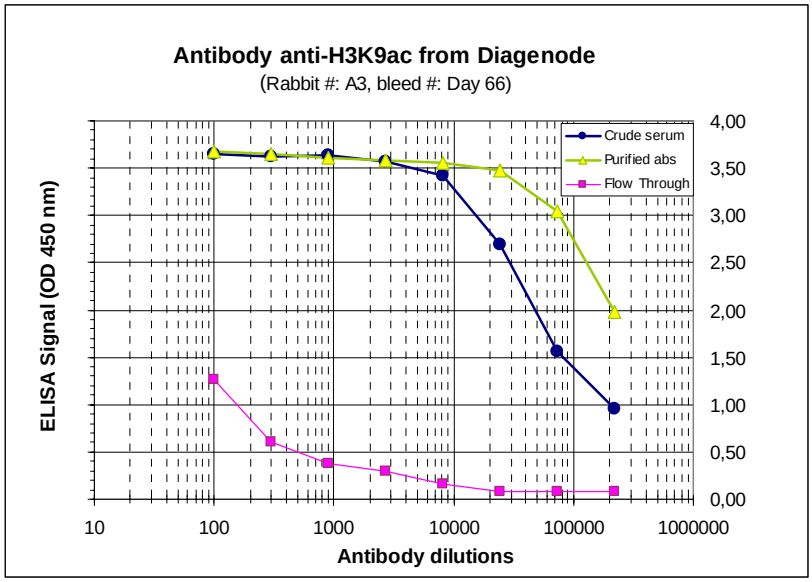
Storage: For long storage, store at -20°C. Do not freeze-thaw.

Precautions: This product is for research use only. Not for use in diagnostic or therapeutic procedures.

Availability date: May 15, 2006. Last data sheet update: April 04, 2008

Lot #: DA-0010: Rabbit #: A3/ bleed #: Day 66/ purification day: December 13, 2006

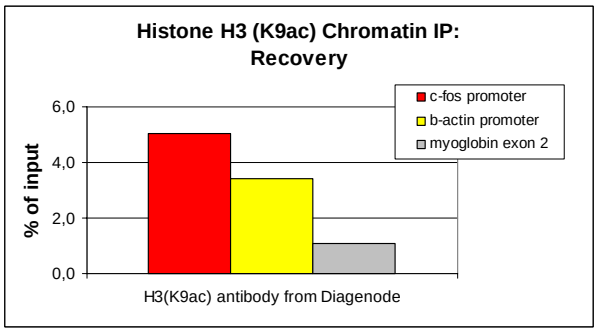
Figure 1:

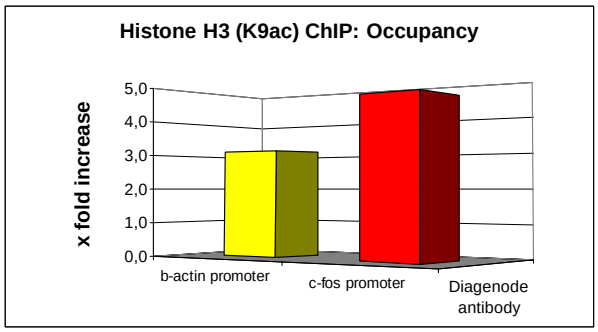


Antibody titer

ELISA, which stands for Enzyme-Linked Immunosorbent Assay, is a quantitative method used to determine the concentration of a primary antibody in sera by using a series of dilutions of sera in antigen coated wells. The antigen used in this case is the peptide including the histone H3 [acetylated lysine 9]. We plotted the absorbance versus antiserum dilution to estimate the titer of the affinity purified antibody: 1:140,000.

Figure 2:





ChIP results obtained with the antibody directed against H3[K9ac] from Diagenode.

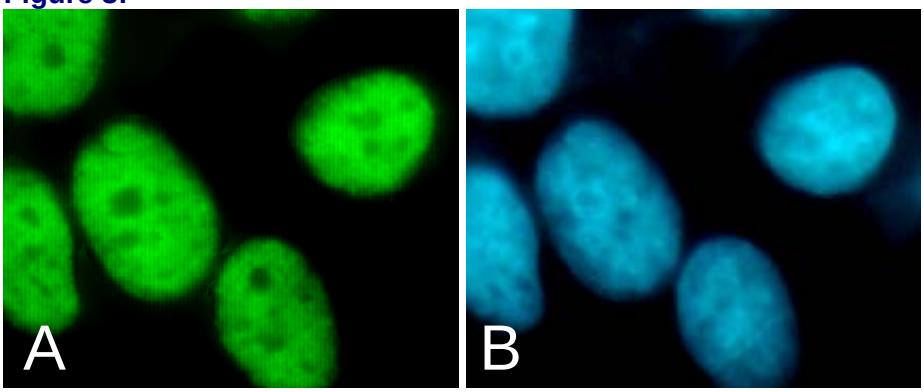
ChIP assays were performed using U2OS cells, the Diagenode antibody directed against histone H3 [K9ac] and optimized PCR primer sets for qPCR. Chromatin sheared from 1x 10e6 cells and 2 µg of antibody anti-H3[K9ac] were used per ChIP experiment. Recovery (%: ChIP/input) and occupancy (x fold: +ve/-ve) are shown here above.
In red: Recovery and occupancy of the c-fos promoter by the histone H3 [K9ac]
In yellow: Recovery and occupancy of b-actin promoter by the histone H3 [K9ac].
Occupancy of the two promoters by the modified histone H3 is evident based on fluorescent qPCR analysis of immunoprecipitated DNA. Controls for IP and PCR specificity include primers for the myoglobin exon 2 (grey, -ve control).

Peptides used in cross-reactivity tests include the following modifications:

H3	H3K9/14ac	H3K9ac	H3K14ac	H4K5/8/12/16ac
-	+	+	-	-

The antibody anti-histone H3 [K9ac] from Diagenode reacts with the peptide including the histone H3 [acetylated lysine 9] modification. Observed cross-reactivity with the tested histone H3 peptides are indicated in the table above, others not tested.

Figure 3:



Immunofluorescence with the antibody anti-histone H3 [K9ac] from Diagenode.

HeLa cells were stained with antibody directed against modified histone and with DAPI. Cells were methanol fixed for 10 minutes and then blocked with 1% BSA containing PBS. The fixation step stabilises the morphology of the cells and permeabilizes membranes as well.

(A) Cells were immunofluorescently labelled with the Diagenode rabbit polyclonal antibody anti-histone H3 [K9ac] (diluted 1:200 and incubated for 1 hour at room temperature) followed by goat anti-rabbit antibody conjugated to FITC.
(B) Nuclei were DAPI stained to label specifically the DNA.

In both cases, the nuclei are clearly stained.