diagenode
Innovating Epigenetic Solutions

ChIP-seq grade

TECHNICAL DATASHEET

HDAC1 polyclonal antibody

Other names: HD1, RPD3, RPD3L1, GON-10

Cat. No. C15410325
Type: Polyclonal **ChIP-grade/ChIP-seq-grade**
Source: Rabbit
Lot #: A21-001P
Size: 50 µg/ 29 µl
Concentration: 1.73 µg/µl

Specificity: Human, mouse: 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 C-terminal region of human HDAC1 (Histone deacetylase 1), using a KLH-conjugated synthetic peptide.

Applications

	Suggested dilution	References
ChIP *	2 µg/ChIP	Fig 1, 2
ELISA	1:4,000	Fig 3
Western blotting	1:1,000	Fig 4, 5
Protein array	1:100,000	Fig 6
Immunofluorescence	1:500	Fig 7

* Please note that the optimal antibody amount per IP should be determined by the end-user. We recommend testing 1-5 µg per IP.

Target description

HDAC1 (UniProt/Swiss-Prot entry Q13547) catalyses the deacetylation of lysine residues on the N-terminal part of the core histones (H2A, H2B, H3 and H4). Acetylation and deacetylation of these highly conserved lysine residues is important for the control of gene expression and HDAC activity is often associated with gene repression. Histone deacetylation is established by the formation of large multiprotein complexes. HDAC1 also interacts with the retinoblastoma tumor suppressor protein and is able to deacetylate p53. Therefore, it also plays an essential role in cell proliferation and differentiation and in apoptosis.

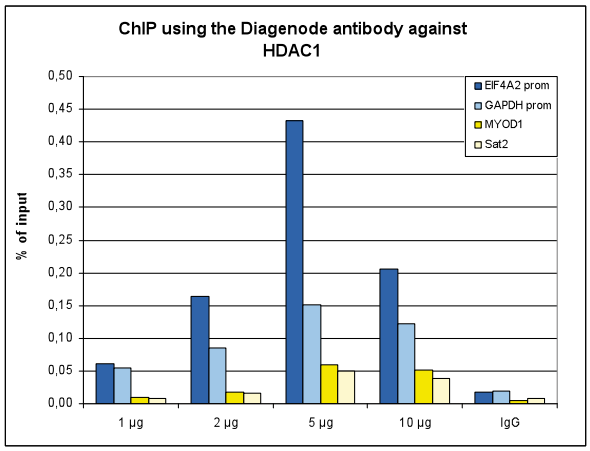

Figure 1. ChIP results obtained with the Diagenode antibody directed against HDAC1

ChIP was performed with the Diagenode antibody against HDAC1 (Cat. No. C15410325) on sheared chromatin from 4,000,000 HeLa cells. An antibody titration consisting of 1, 2, 5 and 10 µg per ChIP experiment was analysed. IgG (2 µg/IP) was used as negative IP control. QPCR was performed with primers specific for the EIF4A2 and GAPDH promoters, used as positive controls, and for the MYOD1 gene and 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).

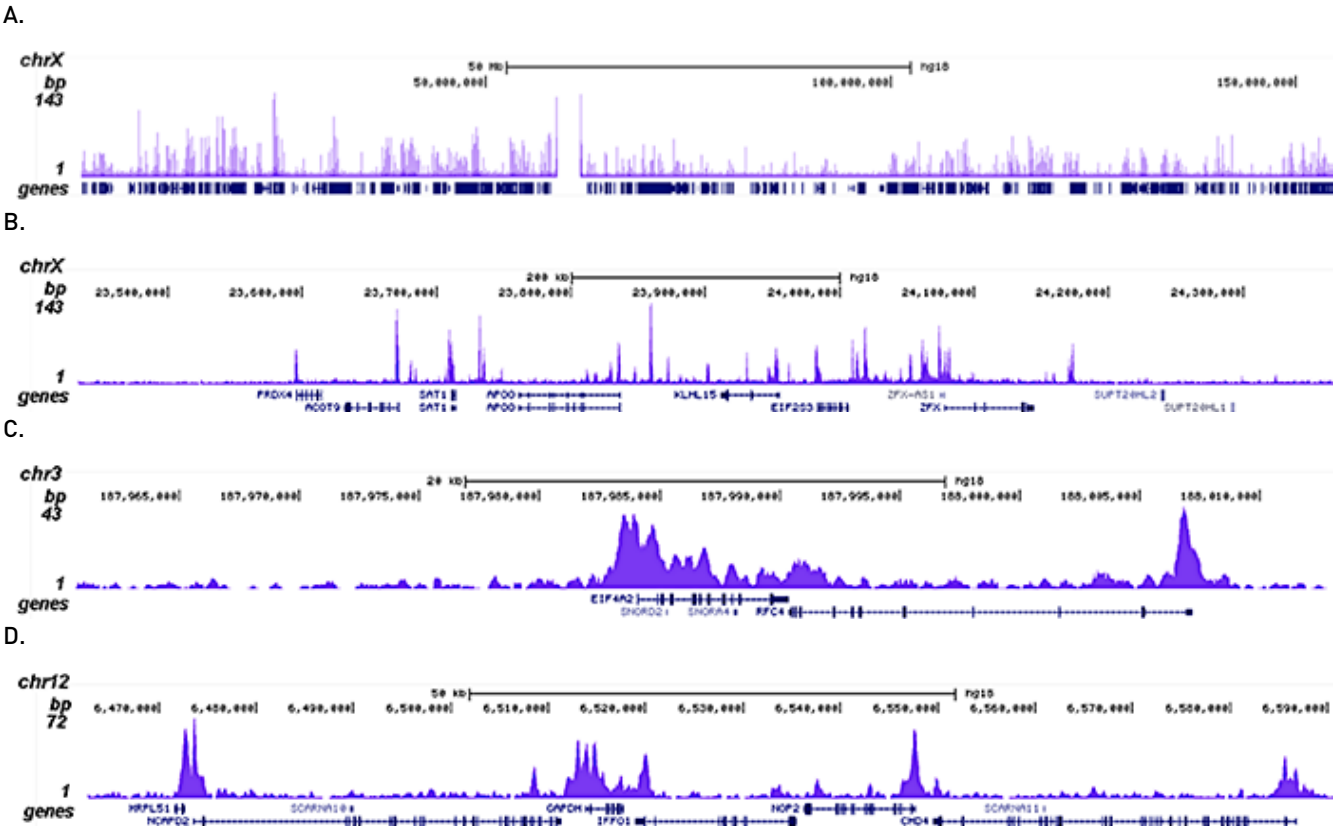

Figure 2. ChIP-seq results obtained with the Diagenode antibody directed against HDAC1

ChIP was performed on sheared chromatin from 4,000,000 HeLa cells using 2 µg of the Diagenode antibody against HDAC1 (Cat. No. C15410325) as described above. The IP'd DNA was subsequently analysed on an Illumina HiSeq 2000. Library preparation, cluster generation and sequencing were performed according to the manufacturer's instructions. The 50 bp tags were aligned to the human genome using the BWA algorithm. Figure 2 shows the peak distribution along the complete sequence and a 1 Mb region of the X-chromosome (figure 2A and B) and in two regions surrounding the GAPDH and EIF4A2 positive control genes, respectively (figure 2C and D).

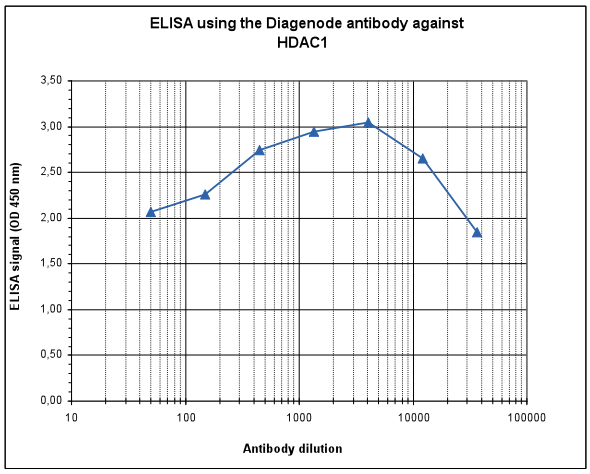

Figure 3. Determination of the antibody titer

To determine the titer of the antibody, an ELISA was performed using a serial dilution of Diagenode antibody directed against HDAC1 (Cat. No. C15410325), crude serum and flow through. The plates were coated with the peptide used for immunization of the rabbit. By plotting the absorbance against the antibody dilution (Figure 2), the titer of the antibody was estimated to be 1:75,000.

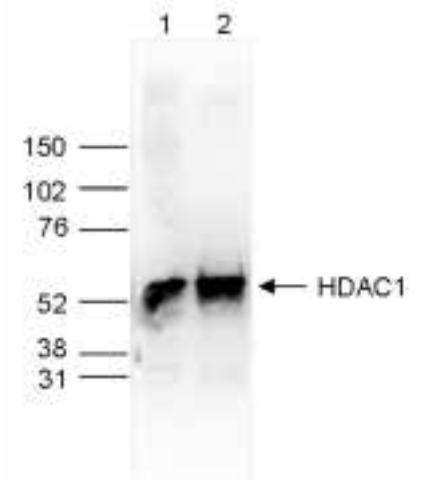

Figure 4. Western blot analysis using the Diagenode antibody directed against HDAC1

Whole cell extracts (25 µg, lane 1) and nuclear extracts (25 µg, lane 2) from HeLa cells were analysed by Western blot using the Diagenode antibody against HDAC1 (Cat. No. C15410325) diluted 1:1,000 in TBS-Tween containing 5% skimmed milk. The position of the protein of interest is indicated on the right (expected size: 55 kDa); the marker (in kDa) is shown on the left.

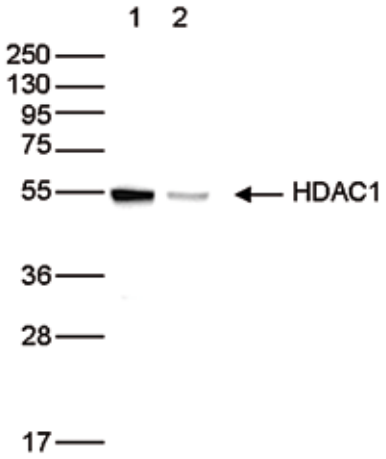

Figure 5. Western blot analysis using the Diagenode antibody directed against HDAC1

Whole cell extracts (50 µg) from HeLa cells transfected with HDAC1 siRNA (lane 2) and from an untransfected control (lane 1) were analysed by Western blot using the Diagenode antibody against HDAC1 (Cat. No. C15410325) diluted 1:1,000 in TBS-Tween containing 5% skimmed milk. The position of the protein of interest is indicated on the right (expected size: 55 kDa); the marker (in kDa) is shown on the left.

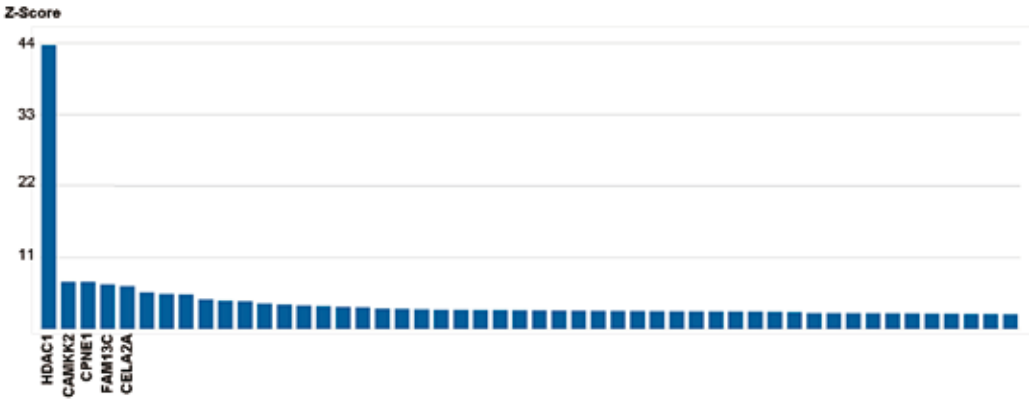

Figure 6. Protein array analysis with the Diagenode antibody directed against HDAC1

The specificity of the Diagenode antibody against HDAC1 (Cat. No. C15410325) was demonstrated using the HuProt human protein microarray (CDI Laboratories), a protein array containing more than 19,000 human proteins. The antibody was used at a dilution of 1:100,000. Figure 6 shows the Z-score of the signal intensity (mean value of the duplicate spots on the array). The names of the proteins with 5 highest Z-scores are indicated at the bottom. This figure clearly shows the high specificity of the antibody for HDAC1.

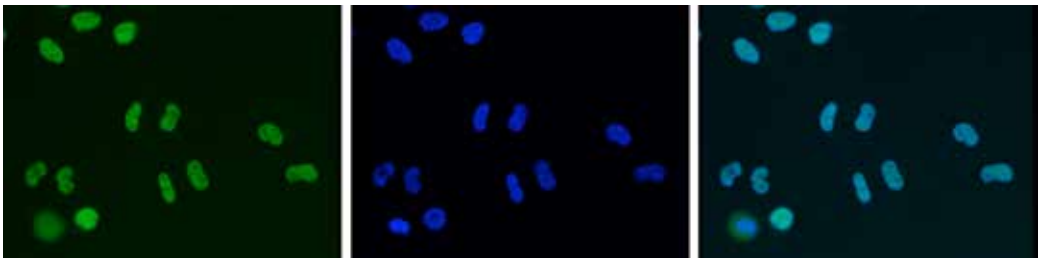

Figure 7. Immunofluorescence using the Diagenode antibody directed against HDAC1

HeLa cells were stained with the Diagenode antibody against HDAC1 (Cat. No. C15410325) 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 HDAC1 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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