diagenode
A Hologic Company

ChIP-seq grade

TECHNICAL DATASHEET

SIN3A polyclonal antibody - Classic

Cat. No. C15410250

Type: Polyclonal

Source: Rabbit **ChIP-grade/ChIP-seq grade**

Lot #: 41507

Size: 25 µl/100 µl

Concentration: 1 µg/µl

Specificity: Human, mouse, rat: positive
Other species: not tested

Purity: Affinity purified polyclonal antibody in PBS containing 20% glycerol and 0.01% thimerosal.

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 SIN3A (SIN3 Transcription Regulator Family Member A), using a recombinant protein.

Applications

	Suggested dilution*	Results
ChIP*	2 µg per ChIP	Figure 1, 2
Western blotting	1:500-1:3,000	Figure 3
Immunoprecipitation	3 µg per IP	Figure 4
Immunofluorescence	1:100-1:1,000	Figure 5
Immunohistochemistry	1:100-1:1,000	Figure 6

* 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

SIN3A (UniProt/Swiss-Prot entry Q96ST3) acts as a transcriptional repressor. It forms a complex with histone deacetylases and also interacts with the transcriptional repression domain of methyl-CpG-binding protein-2. The Sin3a/HDAC complex is critical for cell cycle control and apoptosis throughout development.

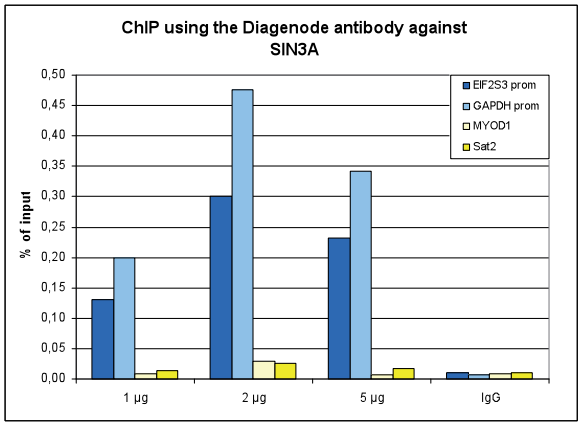

Figure 1. ChIP results obtained with the Diagenode antibody directed against SIN3A

ChIP assays were performed using HeLa cells, the Diagenode antibody against SIN3A (Cat. No. C15410250) and optimized primer sets for qPCR. ChIP was performed with the "iDeal ChIP-seq" kit (Cat. No. C01010055), using sheared chromatin from 4 million cells. A titration of the antibody consisting of 1, 2 and 5 µg per ChIP experiment was analysed. IgG (1 µg/IP) was used as negative IP control. QPCR was performed with primers for the promoters of the GAPDH and EIF2S3 genes, used as positive controls, and for the MYOD1 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).

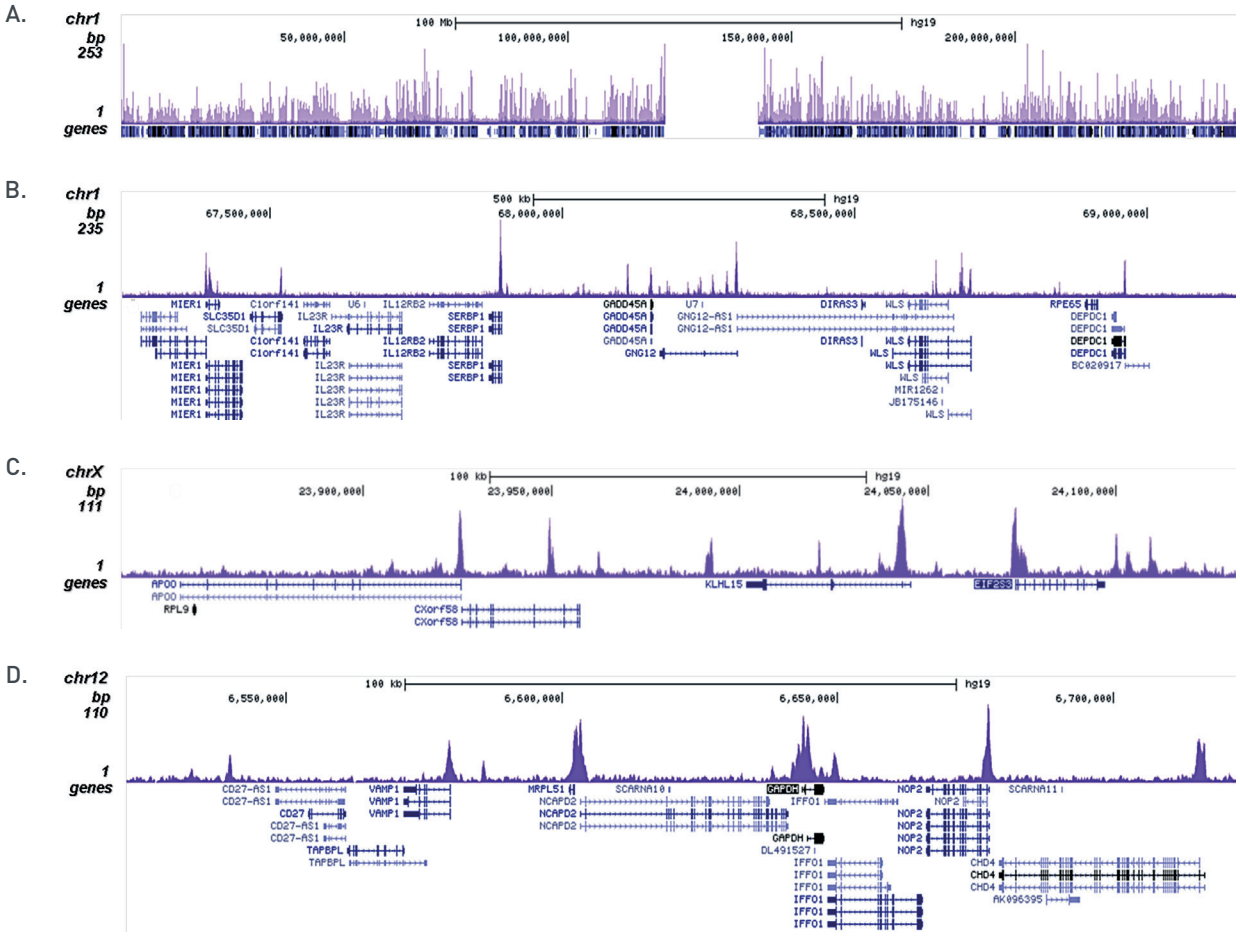

Figure 2. ChIP-seq results obtained with the Diagenode antibody directed against SIN3A

ChIP was performed on sheared chromatin from 4 million HeLa cells using 2 µg of the Diagenode antibody against SIN3A (Cat. No. C15410250) as described above. The IP'd DNA was subsequently analysed on an Illumina HiSeq. 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 enrichment along the complete sequence and a 1.5 Mb region of human chromosome 1 (fig 2A and B), and in two genomic regions surrounding the EIF2S3 and GAPDH positive control genes (fig 2C and D).

Figure 3. Western blot analysis using the Diagenode antibody directed against SIN3A

Whole cell extracts from 293T (lane 1), A431 (lane 2), HeLa (lane 3) and HepG2 (lane 4) cells were analysed by Western blot using the Diagenode antibody against SIN3A (Cat. No. C15410250) diluted 1:1,000. The position of the protein of interest is indicated on the right; the marker (in kDa) is shown on the left.

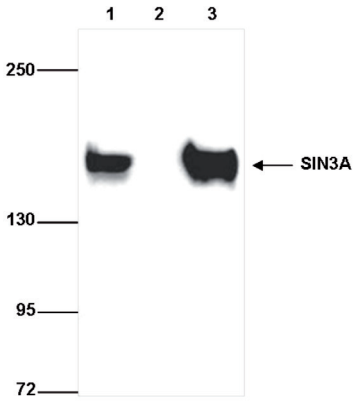

Figure 4. Immunoprecipitation using the Diagenode antibody directed against SIN3A

Immunoprecipitation was performed on whole cell extracts from 293T cells using 3 µg of the Diagenode antibody against SIN3A (Cat. No. C15410250). An equal amount of rabbit IgG was used as a negative control. The immunoprecipitated SIN3A protein was detected by western blot with the SIN3A antibody diluted 1:1,000. The IP with the SIN3A antibody and with the IgG negative control are shown in lane 3 and lane 2, respectively. Lane 1 shows the input (30 µg of 293T whole cell extract).

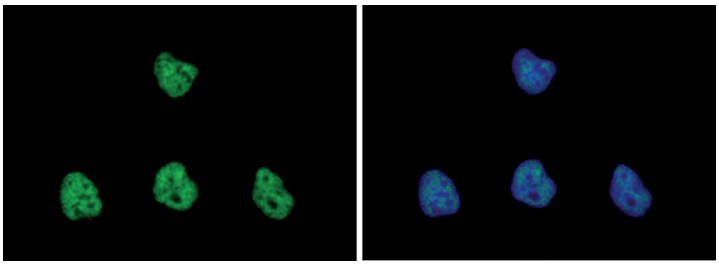

Figure 5. Immunofluorescence with the Diagenode antibody directed against SIN3A

HeLa cells were fixed with formaldehyde and stained with the Diagenode antibody against SIN3A (Cat. C15410250) diluted 1:1,000 (left). The right picture shows costaining with Hoechst 33342 nucleic acid stain.

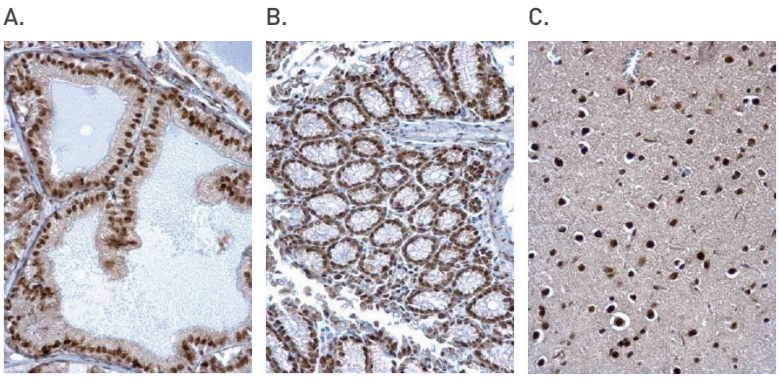

Figure 6. Immunohistochemistry using the Diagenode antibody directed against SIN3A

Formalin fixed paraffin embedded mouse prostate (fig 6A), mouse colon (fig 6B) or rat fore brain (fig 6C) tissue was stained with the Diagenode antibody against SIN3A (Cat. No. C15410250) diluted 1:500 followed by a peroxidase labelled goat anti-rabbit secondary antibody.

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