diagenode
Innovating Epigenetics Solutions

ChIP-seq grade

TECHNICAL DATASHEET

BRD4 polyclonal antibody

Cat. No. C15410337

Type: Polyclonal	Specificity: Human
Isotype: NA	Concentration: 2.6 µg/µl
Source: Rabbit	Purity: Affinity purified polyclonal antibody in PBS containing 0.05% azide and 0.05% ProClin 300.
Lot No.: A2710P	Storage: Store at -20°C; for long storage, store at -80°C. Avoid multiple freeze-thaw cycles.
Size: 50 µg/20 µl	Precautions: This product is for research use only. Not for use in diagnostic or therapeutic procedures.

Last Data Sheet Update: April 26, 2017

Description

Polyclonal antibody raised in rabbit against human BRD4 (Bromodomain Containing 4), using two KLH-conjugated synthetic peptides from the N-terminal and central part of the protein, respectively.

Applications

Applications	Suggested dilution/amount	References
ChIP *	2 µg/ChIP	Fig 1, 2
ELISA	1:10,000	Fig 3
Western blotting	1:1,000	Fig 4

*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

BRD4 (UniProt/Swiss-Prot entry O60885) is a chromatin reader protein that binds acetylated histones. It remains associated with acetylated chromatin throughout the entire cell cycle and provides epigenetic memory for gene transcription by preserving an acetylated chromatin status. As such, it plays a key role in the transmission of epigenetic memory across cell divisions. BRD4 promotes phosphorylation of Ser-2 of the C-terminal domain (CTD) of RNA polymerase II and plays a key role in regulating the transcription of signal-inducible genes. It has been implicated in a translocation of chromosome 19 which causes an upper respiratory tract carcinoma.

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Validation Data

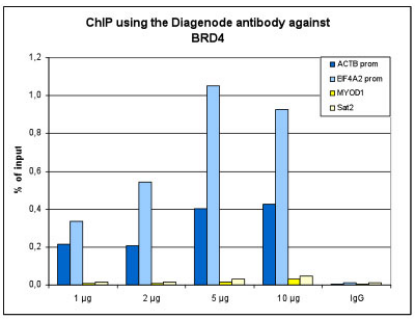

Figure 1. ChIP results obtained with the Diagenode antibody directed against BRD4

ChIP was performed with the Diagenode antibody against BRD4 (Cat. No. C15410337) on sheared chromatin from 4,000,000 K562 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 ACTB 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).

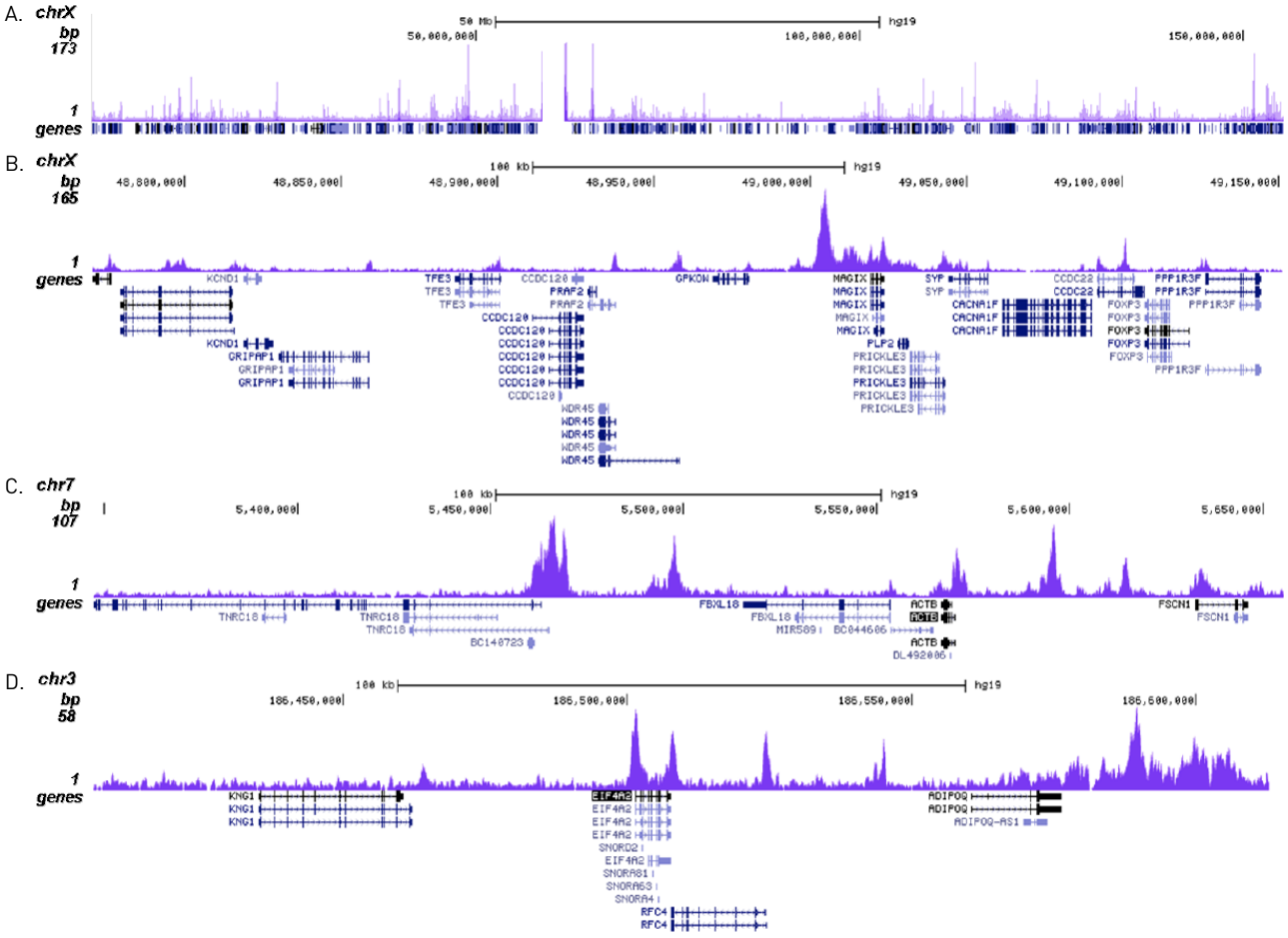

Figure 2. ChIP-seq results obtained with the Diagenode antibody directed against BRD4

ChIP was performed with 2 µg of the Diagenode antibody against BRD4 (cat. No. C15410337) on sheared chromatin from 4,000,000 K562 cells using the "iDeal ChIP-seq" kit 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 signal distribution along the complete sequence and a 300 kb region of the human X-chromosome (figures 2A and B), and in two genomic rtegions surrounding the ACTB and EIF4A2 positive control genes (figure 2C and D).

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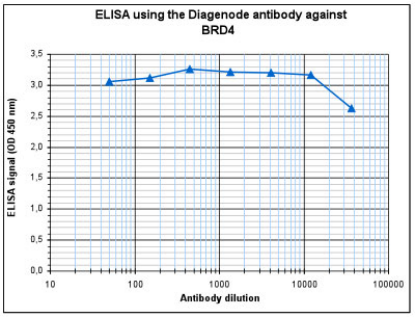

Figure 3. Determination of the antibody titer

To determine the titer of the antibody, an ELISA was performed using a serial dilution of the Diagenode antibody directed against BRD4 (Cat. No. C15410337). The plates were coated with the peptides 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 1:290,000.

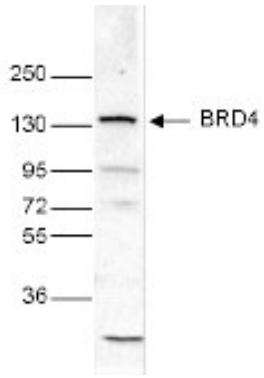

Figure 4. Western blot analysis using the Diagenode antibody directed against BRD4

Whole cell extracts (25 µg) from HeLa cells were analysed by Western blot using the Diagenode antibody against BRD4 (Cat. No. C15410337) 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.

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