diagenode
A Hologic Company

TECHNICAL DATASHEET

CBX8 Antibody - ChIP-seq Grade

Cat. No. C15410333

Type: Polyclonal **ChIP grade, ChIP-seq grade**	Specificity: Human: positive. Other species: not tested.
Size: 100 µl	Isotype: NA
Concentration: 1 µg/µl	Host: Rabbit
Lot No.: A300-882A2	Purity: Affinity purified polyclonal antibody.
Storage buffer: Tris-citrate/phosphate buffer containing 0.09% azide.	Storage conditions: Store at 4°C
Precautions: This product is for research use only. Not for use in diagnostic or therapeutic procedures.	

Last Data Sheet Update: January 13, 2017

Description

Polyclonal antibody raised in rabbit against human **CBX8 (chromobox 8)**, using a synthetic peptide containing a sequence from the C-terminal part of the protein.

1 Manufactured by Bethyl Laboratories, Inc., Texas, USA

Applications

Applications	Suggested dilution	References
ChIP *	1-2 µg/ChIP	Fig 1, 2
Western blot	1:1,000	Fig 3
IP	6 µg per IP	Fig 4

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

Target Description

CBX8 (UniProtKB/Swiss-Prot entry Q9HC52) is a component of the polycomb group PRC1-like multiprotein complex. This complex is required to maintain the transcriptionally repressive state of many genes, including Hox genes, throughout development. It mediates monoubiquitination of histone H2A Lys-119, which leads to recruitment of PRC2 and methylation of H3K27 and H3K9.

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Validation data

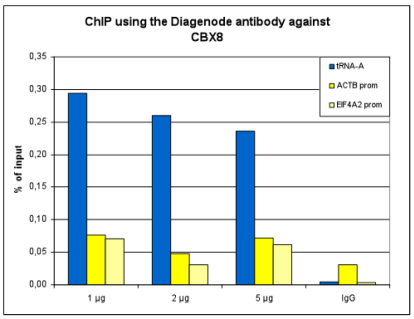

Figure 1. ChIP results obtained with the Diagenode antibody directed against CBX8

ChIP assays were performed using K562 cells, the Diagenode antibody against CBX8 (Cat. No. C15410333) and optimized PCR 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 consisting of 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 for, the tRNA-Ala gene, used as positive control, and for the promoters of the active ACTB and EIF4A2 genes, 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).

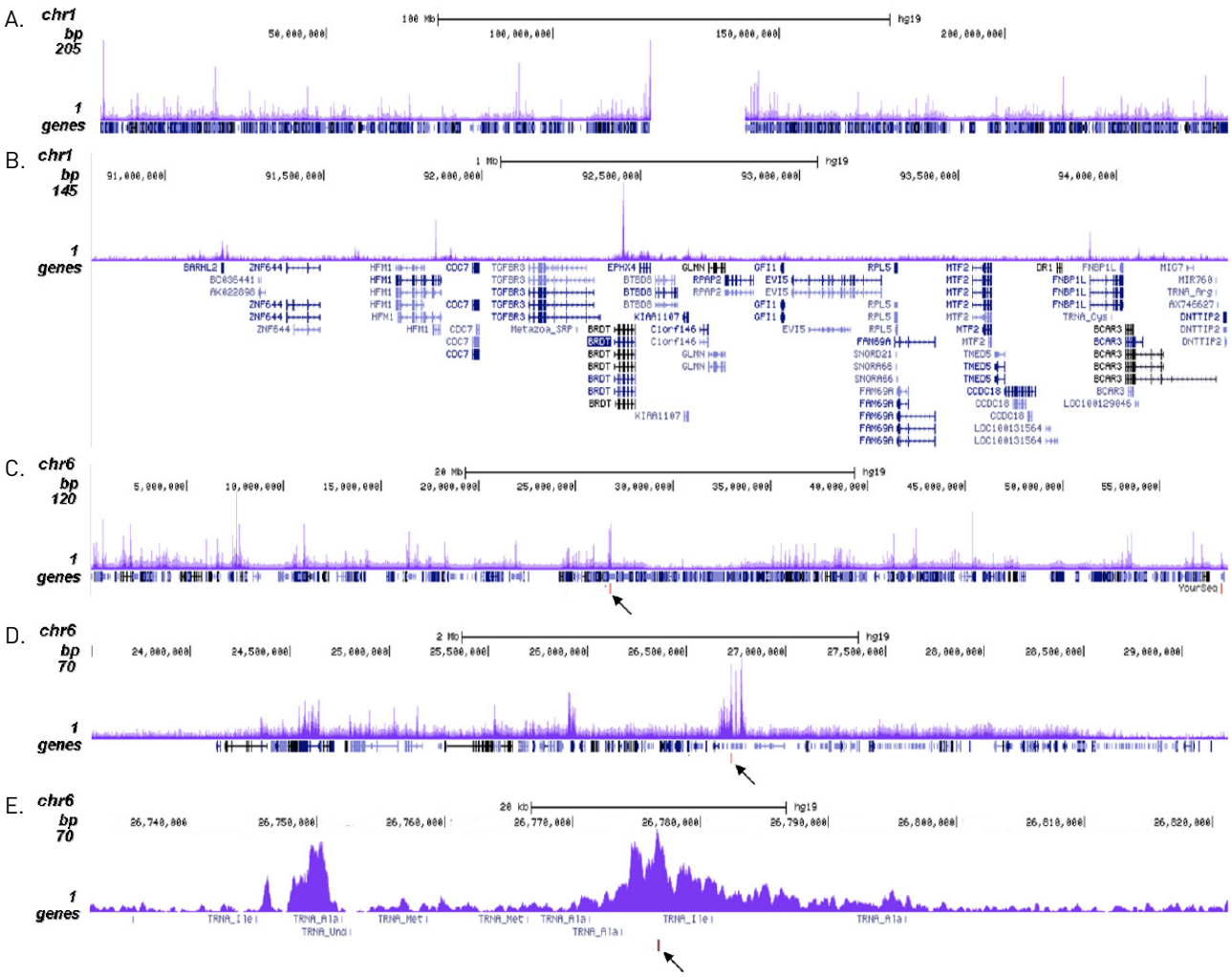

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Figure 2. ChIP-seq results obtained with the Diagenode antibody directed against CBX8

ChIP was performed on sheared chromatin from 4 million K562 cells using 2 µg of the Diagenode antibody against CBX8 (Cat. No. C15410333) 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 3 Mb region of the human chromosome 1 (fig 2A and B), and along the short arm and two zoomins of chromosome 6 (fig 2C and D). The position of the amplicon used for ChIP-qPCR validation is indicated by an arrow.

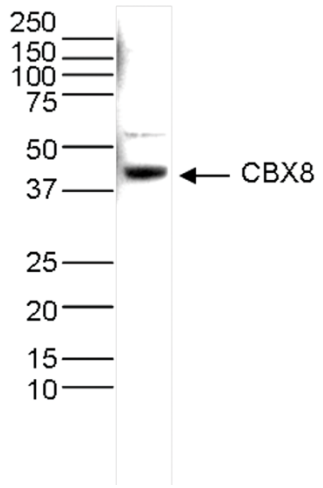

Figure 3. Western blot analysis using the Diagenode antibody directed against CBX8

Whole cell extracts from HeLa cells were analysed by Western blot using the Diagenode antibody against CBX8 (Cat. No. C15410333) 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.

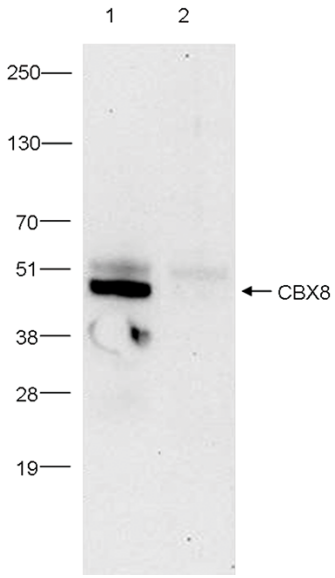

Figure 4. Immunoprecipitation analysis using the Diagenode antibody directed against CBX8

Immunoprecipitation was performed on whole cell extracts from HeLa cells using 6 µg of the Diagenode antibody against CBX8 (Cat. No. C15410333, lane 1). An equal amount of rabbit IgG was used as a negative control (lane 2). The immunoprecipitated CBX8 protein was detected by western blot with the CBX8 antibody diluted 1:2,500.

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