diagenode
A Hologic Company

TECHNICAL DATASHEET

EZH2 polyclonal antibody

Cat. No. C15410039-50

Type: Polyclonal	Specificity: Human, mouse
Size: 50 µg	Isotype: NA
Concentration: 1.0 µg/µl	Host: Rabbit
Lot No.: 003	Purity: Protein G purified
Storage buffer: NA	Storage conditions: NA
Precautions: This product is for research use only. Not for use in diagnostic or therapeutic procedures.	

Last Data Sheet Update: January 17, 2017

Description

Other names: ENX-1, ENX1, KMT6, KMT6A, WVS, WVS2

Polyclonal antibody raised in rabbit against the N-terminus (aa1-343) of the mouse EZH2 protein (Enhancer of zeste homolog 2).

Applications

Applications	Suggested dilution	References
ChIP *	2 µg/ChIP	Fig 1, 2
Western Blotting	1:1,000	Fig 3, 4
Immunofluorescence	1:1000	Fig 5

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

Target Description

EZH2 (UniProt/Swiss-Prot entry Q15910) is a histone-lysine methyltransferase which methylates ‘Lys-9’ and ‘Lys-27’ of histone H3, leading to transcriptional repression. It is a member of the polycomb group (PcG) family which form multimeric protein complexes and are involved in maintaining the transcriptional repressive state of genes over successive cell generations. The EZH2 activity is dependent on the association with other components of the PRC2 complex (EED, EZH2, SUZ12/JJAZ1, RBBP4 and RBBP7). EZH2 may play a role in the hematopoietic and central nervous systems. Over-expression of EZH2 is observed during advanced stages of prostate cancer and breast cancer.

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Validation Data

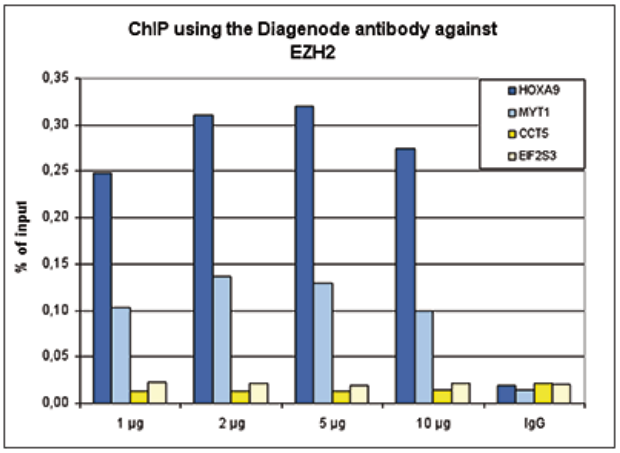
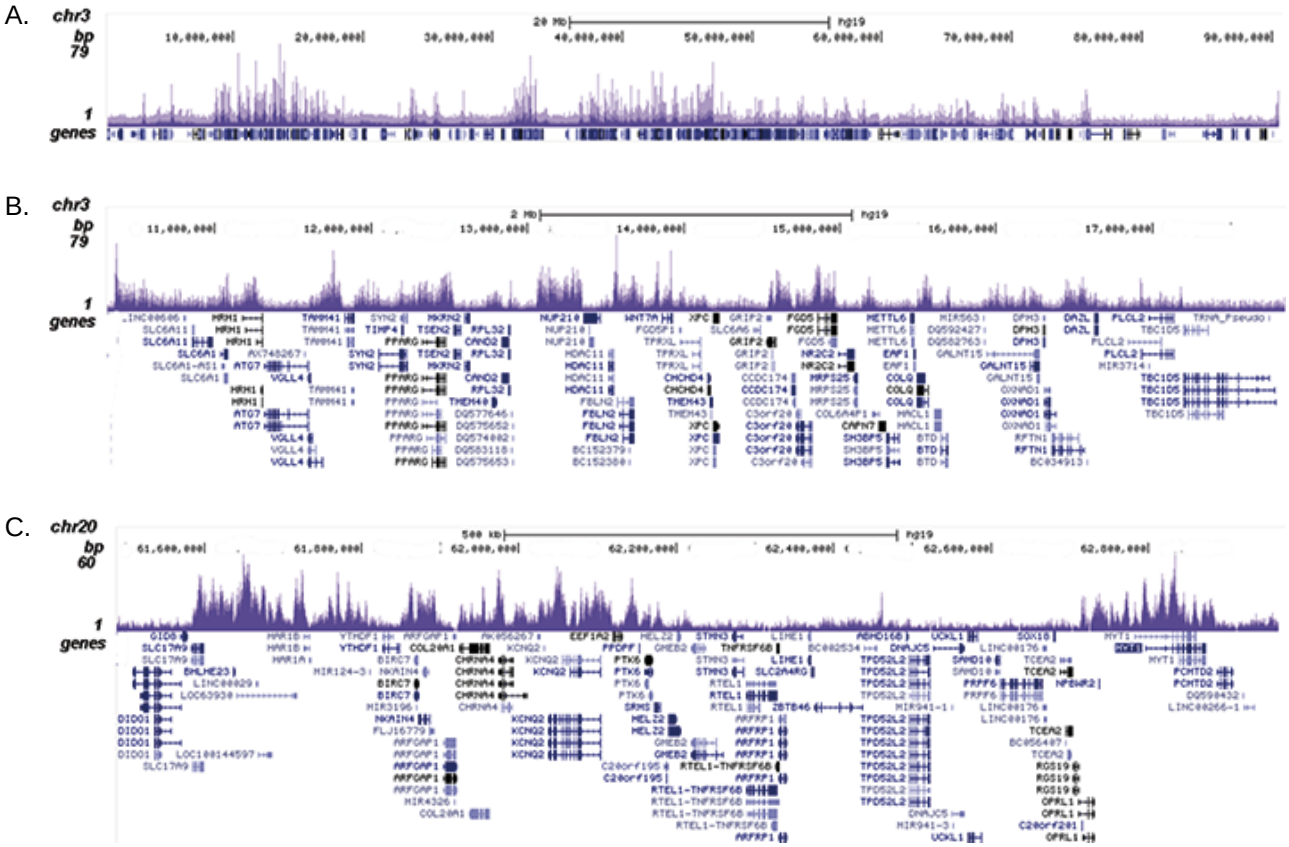


Figure 1. ChIP results obtained with the Diagenode antibody directed against EZH2

ChIP assays were performed using K562 cells, the Diagenode antibody against EZH2 (Cat. No. C15410039) 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 of the antibody consisting of 1, 2. 5 and 10 µg per ChIP experiment was analysed. IgG (2 µg/IP) was used as negative IP control. Quantitative PCR was performed with primers for MYT1 and HOXA9, used as positive control targets, and for the coding regions of the active CCT5 and EIF2S3 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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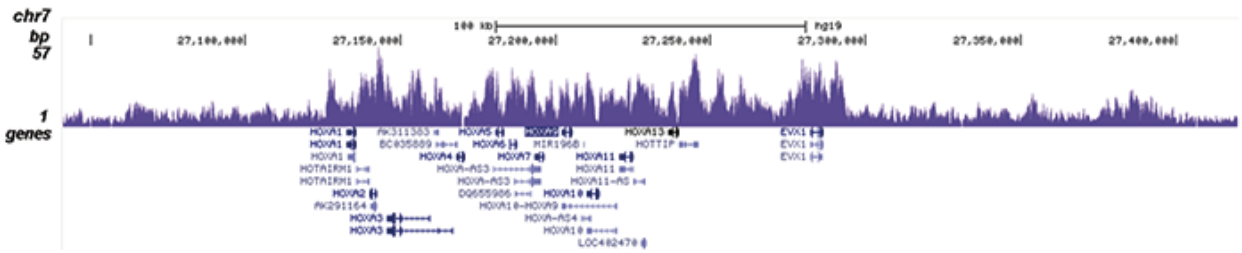

Figure 2. ChIP-seq results obtained with the Diagenode antibody directed against EZH2

ChIP was performed on sheared chromatin from 4 million K562 cells using 2 µg of the Diagenode antibody against EZH2 (Cat. No. C15410039) 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 51 bp tags were aligned to the human genome using the BWA algorithm. Figure 2 shows the peak distribution along the short arm and a 6 Mb region containing several enriched regions of human chromosome 3 (figure 2A and B, respectively), and in two genomic regions containing the MYT1 gene on chromosome 20 and the HOX cluster on chromosome 7 (figure 2C and D).

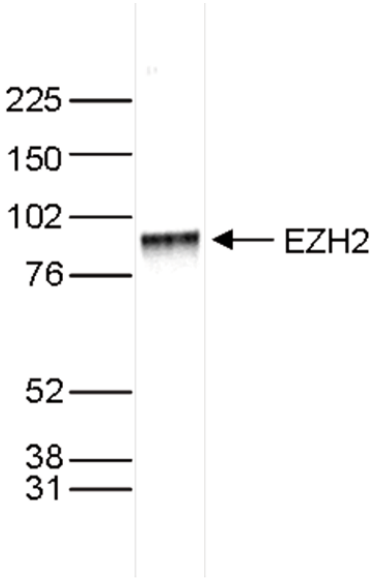

Figure 3. Western blot analysis using the Diagenode antibody directed against EZH2

Nuclear extracts of HeLa cells (40 µg) were analysed by Western blot using the Diagenode antibody against EZH2 (Cat. No. C15410039) diluted 1:1,000 in TBS-Tween containing 5% skimmed milk. The position of the protein of interest (expected size 85 kDa) is indicated on the right; the marker (in kDa) is shown on the left.

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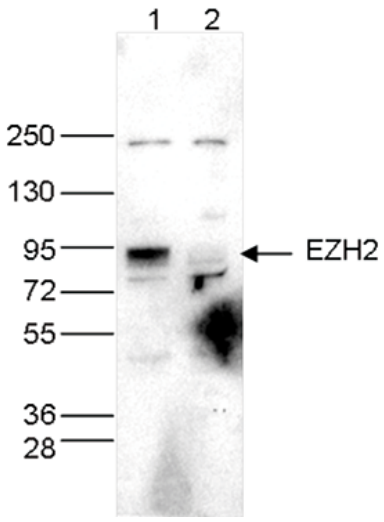

Figure 4. Western blot analysis using the Diagenode antibody directed against EZH2

Whole cell extracts (40 µg) from HeLa cells transfected with EZH2 siRNA (lane 2) and from an untransfected control (lane 1) were analysed by Western blot using the Diagenode antibody against EZH2 (Cat. No. C15410039) 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.

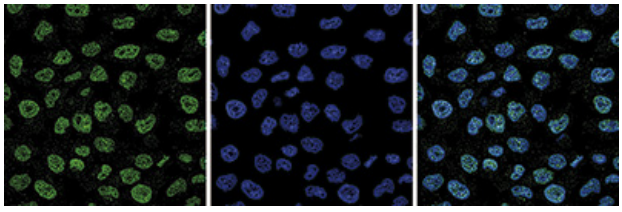

Figure 5. Immunofluorescence using the Diagenode antibody directed against EZH2

HeLa cells were stained with the Diagenode antibody against EZH2 (cat. No. C15410039) and with DAPI. Cells were fixed with 4% formaldehyde for 10' and blocked with PBS/TX-100 containing 1% BSA. The cells were immunofluorescently labelled with the EZH2 antibody (left) diluted 1:1,000 in blocking solution followed by an anti-mouse 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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