diagenode
A Hologic Company

TECHNICAL DATASHEET

AML1-ETO polyclonal antibody

Other names: RUNX1-RUNX1T1 fusion protein

Cat. No. C15310197

Type: Polyclonal
ChIP-grade / ChIP-seq-grade

Source: Rabbit

Lot #: A706-001

Size: 100 µl

Concentration: not determined

Specificity: Human: positive
Other species: not tested

Purity: Whole antiserum from rabbit containing 0.05% azide.

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 AML1-ETO fusion protein using a KLH-conjugated synthetic peptide.

Applications

	Suggested dilution	Results
ChIP*	4 µl/ChIP	Fig 1, 2
ELISA	1:500	Fig 3
Western blotting	1:1,000	Fig 4

* Please note that the optimal antibody amount per ChIP should be determined by the end-user. We recommend testing 1-10 µl per IP.

References citing this antibody:

(1) Martens JHA, Mandoli A, Simmer F, Wierenga B-J, Saeed S, Singh AA, Altucci L, Vellenga E, Stunnenberg HG (2012) ERG and FLI1 binding sites demarcate targets for aberrant epigenetic regulation by AML1-ETO in acute myeloid leukemia. Blood 120: 4038-4048.

Target description

This antibody specifically recognizes the AML1 (RUNX1) (UniProtKB/Swiss-Prot entry Q01196) - ETO (RUNX1T1) (UniProtKB/Swiss-Prot entry Q06455) fusion protein that arises due to a translocation between chromosome 8 and 22 (t(8;21)(q22;q22)). This translocation is one of the most frequent karyotypic abnormalities observed in acute myeloid leukaemia. It produces a chimerical gene made up of the 5'-region of AML1and the 3'-region of ETO. The chimerical protein is thought to associate with the nuclear corepressor/histone deacetylase complex to block hematopoietic differentiation.

Results

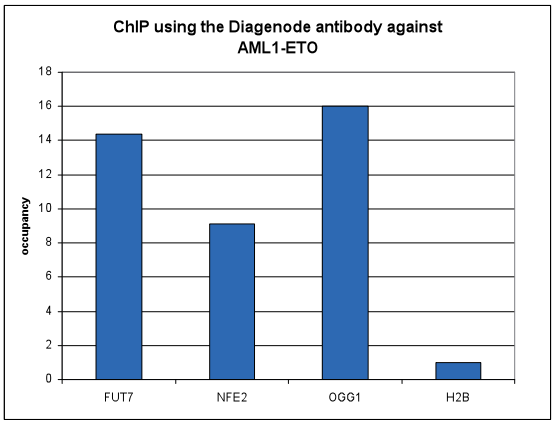
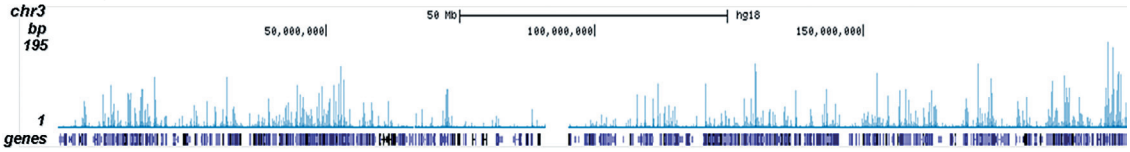


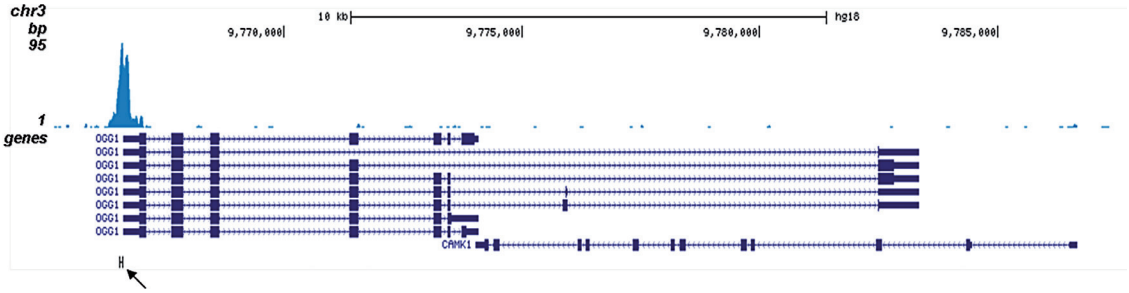
Figure 1. ChIP results obtained with the Diagenode antibody directed against AML1-ETO

ChIP assays were performed using Kasumi-1 cells, the Diagenode antibody against AML1-ETO (Cat. No. C15310197) and optimized primer pairs for qPCR. Sheared chromatin from 1.25 million cells and 4 µl of antibody were used per ChIP experiment. QPCR was performed using primers specific for the FUT7, NFE2 and OGG1 genes. Figure 1 shows the occupancy, calculated as the ratio + control/background for which the promoter of the H2B gene was used.

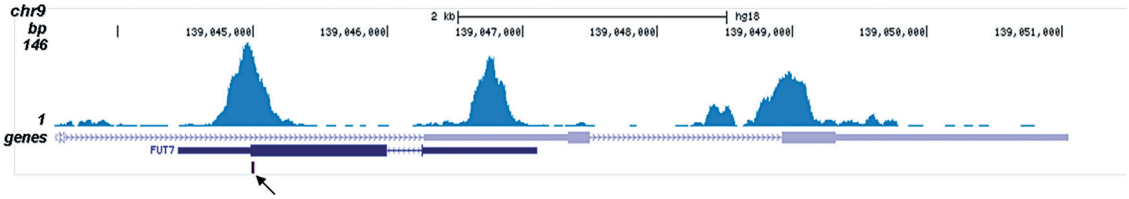
A. ChIP-seq signals on chromosome 3



B. Genomic region on chromosome 3 surrounding the OGG1 gene



C. Genomic region on chromosome 9 surrounding the FUT7 gene



D. Genomic region on chromosome 12 surrounding the NFE2 gene

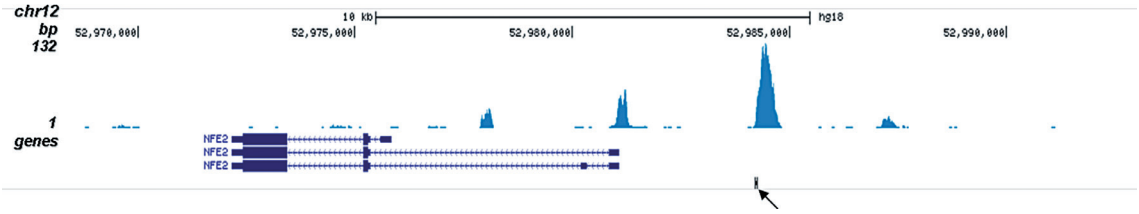


Figure 2. ChIP-seq results obtained with the Diagenode antibody directed against AML1-ETO

ChIP was performed as described above. The IP'd DNA of 6 ChIP's was pooled and analysed with an Illumina Genome Analyzer. Library preparation, cluster generation and sequencing were performed according to the manufacturer's instructions. The 32 bp tags were aligned to the human reference genome (hg18) using the ELAND algorithm. Figure 2 shows the results of the complete chromosome 3 and three genomic regions surrounding the OGG1, FUT7 and NFE2 genes, respectively. The position of the PCR amplicon is indicated with an arrow.

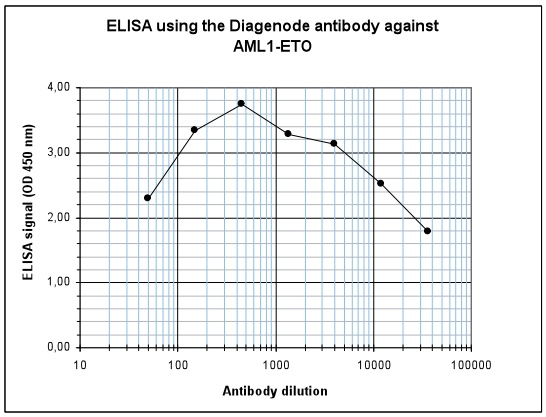


Figure 3. Determination of the antibody titer

To determine the titer of the antibody, an ELISA was performed using a serial dilution of the Diagenode antibody directed against AML1-ETO (Cat. No. C15310197). The plates were coated with the peptides used for immunization of the rabbit. By plotting the absorbance against the antibody dilution (Figure 3), the titer of the antibody was estimated to be 1:32,750.

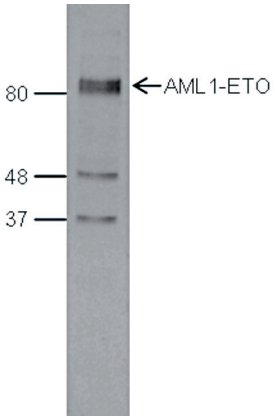


Figure 4. Western blot analysis using the Diagenode antibody directed against AML1-ETO

Nuclear extracts of SKNO-1 cells (15 µg) were analysed by Western blot using the Diagenode antibody against AML1-ETO (Cat. No. C15310197) diluted 1:1,000 in TBS-Tween containing 5% skimmed milk. The marker (in kDa) is shown on the left, the position of the protein of interest is indicated on the right.

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Last update: October 11, 2012