diagenode
A Hologic Company

TECHNICAL DATASHEET

RARA antibody

Cat. No. C15310155

Type: Polyclonal **ChIP grade/ChIP-seq grade**
Source: Rabbit
Lot: A704-002
Size: 100 µl
Concentration: not determined

Specificity: Human, mouse: 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 human RARA (Retinoic Acid Receptor alpha) using two KLH-conjugated synthetic peptides containing sequences from the C-terminal region of the protein.

Applications

Applications	Suggested dilution	References
ChIP*	4 µg per ChIP	Fig 1, 2
ELISA	1:100 - 1:500	Fig 3
Western blotting	1:750	Fig 4

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

Target description

RARA (UniProtKB/Swiss-Prot entry P10276) is a receptor for retinoic acid, a vitamin A metabolite, which directly regulates gene expression in target cells by binding to specific DNA response elements. In the absence of its ligand, this receptor represses transcription through the recruitment of specific corepressors and of HDAC's, whereas binding of retinioc acid causes the recruitment of coactivators and HAT's. Translocations involving the RARA gene, often leading to a RARA/PML fusion protein, are a major cause of acute promyelocytic leukemia.

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Results

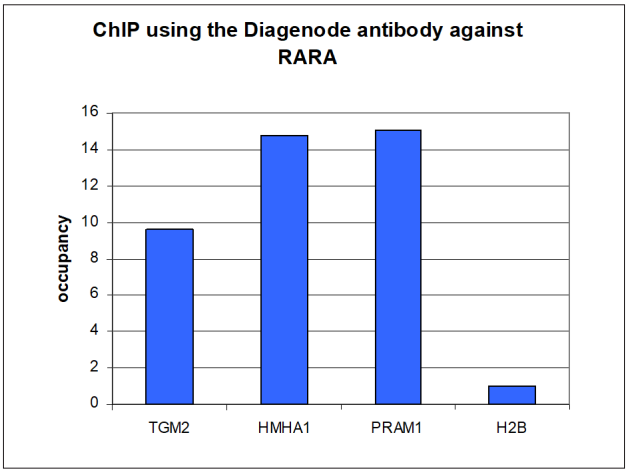
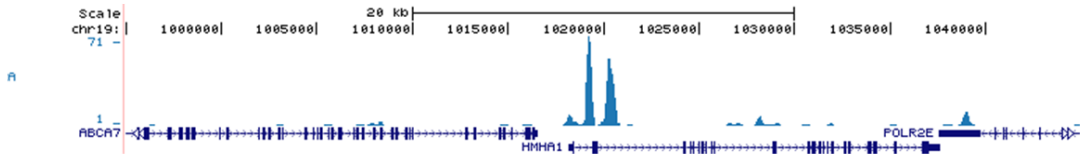


Figure 1. ChIP results obtained with the Diagenode antibody directed against RARA

ChIP assays were performed using NB4 cells, the Diagenode antibody against RARA (cat. No. C15310155) and optimized primer pairs for qPCR. Sheared chromatin from 6 million cells and 4 µl of antibody were used per ChIP experiment. QPCR was performed using primers specific for the TGM2, HMHA1, PRAM1 and H2B genes. Figure 1 shows the relative occupancy, calculated as the ratio + control/background for which the second exon of the MB gene was used.

A. ChIP-seq signals on chromosome 19

B. 50 kb region on chromosome 19 surrounding the HMHA1 gene



C. 50 kb region on chromosome 19 surrounding the PRAM1 gene

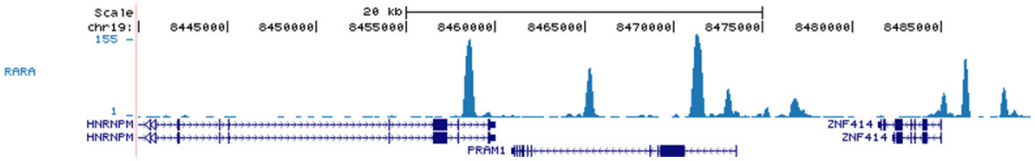


Figure 2. ChIP-seq results obtained with the Diagenode antibody directed against RARA

ChIP was performed as described above and the IP'd DNA was 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 19 and two 50 kb region surrounding the HMHA1 and PRAM1 genes, respectively.

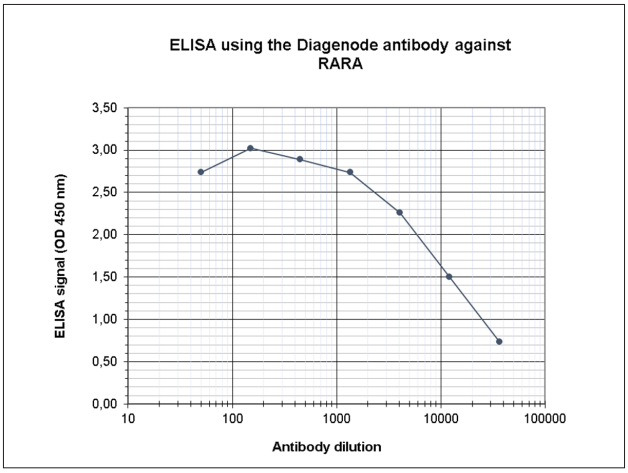


Figure 3. Determination of the antibody titer

To determine the titer of the antibody, an ELISA was performed using a serial dilution of the Diagenode antibody directed against human RARA (cat. No. C15310155). 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 1:11,300.

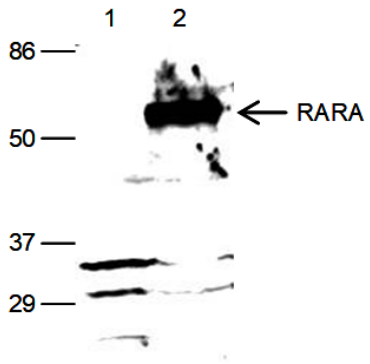


Figure 4. Western blot analysis using the Diagenode antibody directed against RARA

Human embryonic kidney cells (293T) were transfected with a RARA construct (lane 2) or with a negative control construct (lane 1) and analysed by Western blot using the Diagenode antibody against RARA (cat. No. C15310155), diluted 1:750 in BSA/PBS-Tween. The molecular weight marker (in kDa) is shown on the left; the location of the protein of interest is indicated on the right.