

RBBP5 polyclonal antibody

Other names: RBBP-5, RBQ3, SWD1

Cat. No. C15410342

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

Source: Rabbit

Lot #: A300-109A2

Size: 100 µl

Concentration: 1 µg/µl

Specificity: Human: positive

Other species: not tested

Purity: Affinity purified polyclonal antibody in Tris-citrate buffer containing 0.09% azide.

Storage: Store at 4°C.

Precautions: This product is for research use only. Not for use in diagnostic or therapeutic procedures.

Description: Polyclonal antibody raised in rabbit against human RBBP5 (RB Binding Protein 5), using a synthetic peptide containing a sequence from the C-terminus of the protein¹.

Applications

Applications	Suggested dilution	References
ChIP*	2 µg per ChIP	Fig 1, 2
Western blotting	1:1,000	Fig 3
IP	10 µg per IP	Fig 4
IHC	1:1,000	Fig 5

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

Target description

RBBP5 (UniProtKB/Swiss-Prot entry Q15291) belongs to the highly conserved family of WD-repeat proteins. It's part of the MLL1/MLL complex and is as such involved in mono-, di- and trimethylation at lysine 4 of histone H3, a specific tag for epigenetic transcriptional activation. RBBP5 binds to the retinoblastoma protein thereby playing a role in the regulation of cell proliferation. It also plays a crucial role in the differentiation potential in embryonic stem (ES) cells.

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

Results

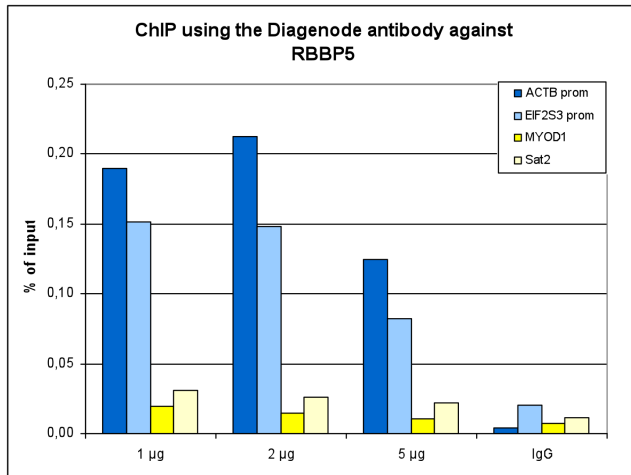


Figure 1. ChIP results obtained with the Diagenode antibody directed against RBBP5

ChIP assays were performed using K562 cells, the Diagenode antibody against RBBP5 [Cat. No. C15410342] 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 promoters of the ACTB and EIF2S3 genes, used as positive controls, and for the MYOD1 gene and the 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)

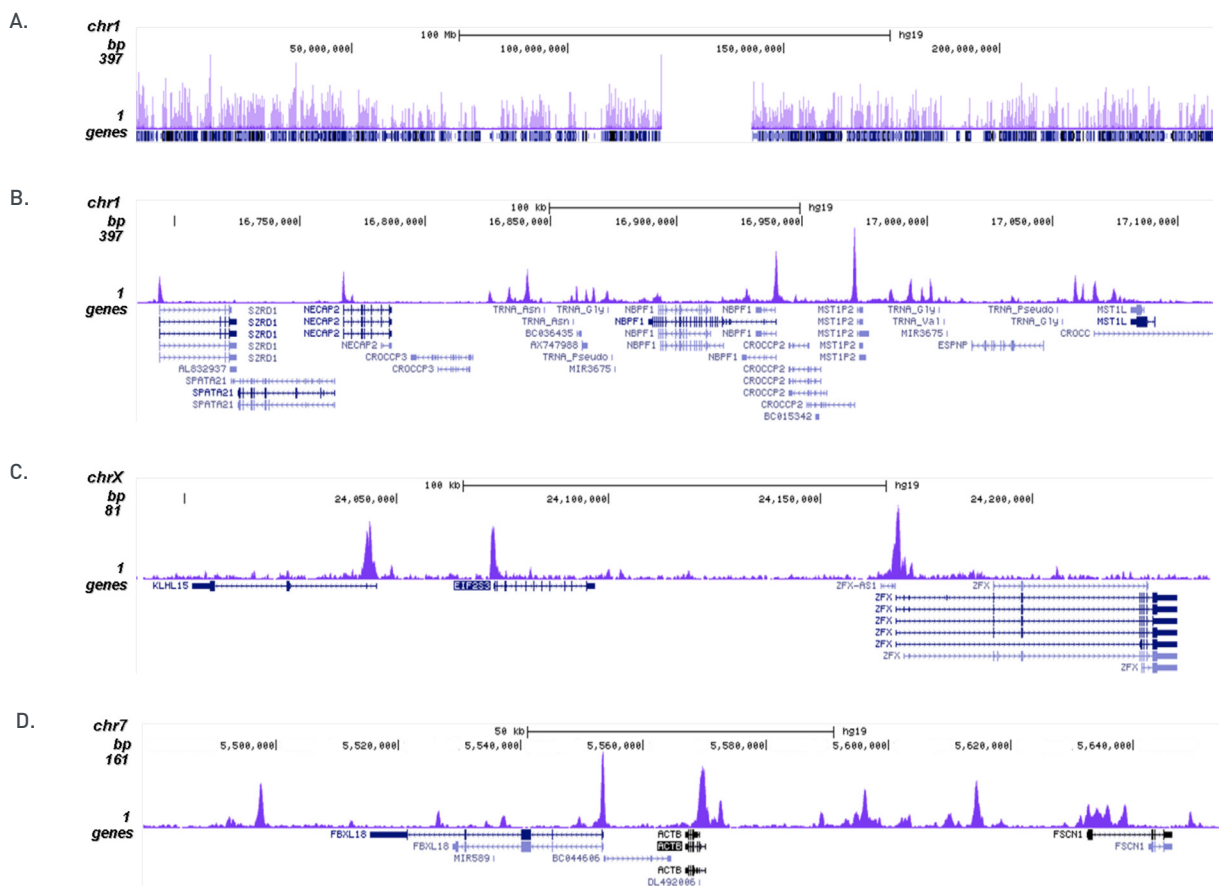


Figure 2. ChIP-seq results obtained with the Diagenode antibody directed against RBBP5

ChIP was performed on sheared chromatin from 4 million K562 cells using 2 µg of the Diagenode antibody against RBBP5 (Cat. No. C15410342) 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 300 kb region of human chromosome 1 (fig 2A and B), and in two genomic regions surrounding the ACTB and EIF2S3 positive control genes (fig 2C and D).

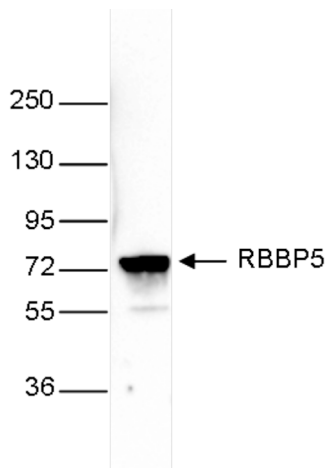


Figure 3. Western blot analysis using the Diagenode antibody directed against RBBP5

Whole cell extracts from HeLa cells were analysed by Western blot using the Diagenode antibody against RBBP5 (Cat. No. C15410342) 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.

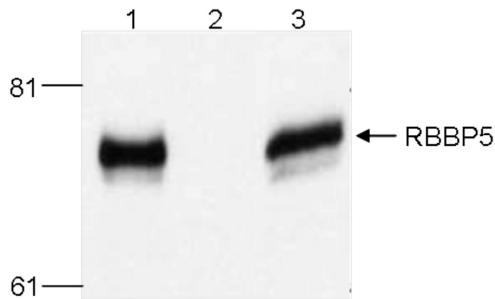


Figure 4. Immunoprecipitation using the Diagenode antibody directed against RBBP5

Immunoprecipitation was performed on whole cell extracts from 293T cells using 10 µg of the Diagenode antibody against RBBP5 (Cat. No. C15410342, lane 1). An equal amount of rabbit IgG was used as a negative control (lane 2). The immunoprecipitated CBX2 protein was detected by western blot with the RBBP5 antibody diluted 1:500. Lane 3 shows the input (15% of the IP).

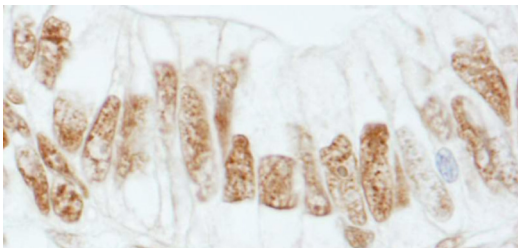


Figure 5. Immunohistochemistry using the Diagenode antibody directed against RBBP5

FFPE sections of human colon carcinoma tissue were analysed by Immunohistochemistry with the Diagenode antibody against RBBP5 (Cat. No. C15410342, lane 1) diluted 1:1,000.

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