

KAP1 polyclonal antibody - Classic

Other names: KAP-1, TRIM28, KRIP-1, RNF96, TIF1B, TF1B, TIF1beta

Cat. No. C15410236

Type: Polyclonal ChIP-grade, ChIP-seq grade

Source: Rabbit

Lot #: 39911

Size: 25 µl/100 µl

Concentration: 1 µg/µl

Specificity: Human, mouse: positive

Other species: not tested

Purity: Affinity purified polyclonal antibody in 100 mM Tris containing 0.1 M Glycin, 10% glycerol and 0.01% thimerosal.

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 KAP1 (KRAB-Associated Protein 1), using a recombinant protein.

Applications

	Suggested dilution*	Results
ChIP*	2 µg/ChIP	Fig 1, 2
Western blotting	1:1,000 - 1:10,000	Fig 3, 4
Immunoprecipitation	2 µg per IP	Fig 5
Immunofluorescence	1:100-1:1,000	Fig 6
Immunohistochemistry	1:100-1:1,000	Fig 7

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

Target description

KAP1 (UniProt/Swiss-Prot entry Q13263) is a member of the tripartite motif family which contain 3 zinc-binding domains, a RING, a B-box type 1 and a B-box type 2, and a coiled-coil region. It mediates transcriptional repression by interaction with the KRAB domain found in many transcription factors. Gene silencing is established by recruiting CHD3, a subunit of the nucleosome remodeling and deacetylation (NuRD) complex, and SETDB1. This results in methylation of H3K9 and deacetylation of H3K9 and H3K14. KAP1 is an important regulator of CDKN1A and mediates the nuclear localization of KRX1, ZNF268 and ZNF300 transcription factors.

Results

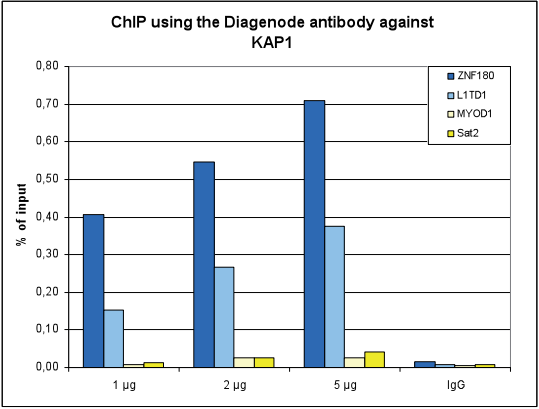


Figure 1. ChIP results obtained with the Diagenode antibody directed against KAP1

ChIP assays were performed using HeLa cells, the Diagenode antibody against KAP1 (Cat. No. C15410236) and optimized 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 and 5 µg per ChIP experiment was analysed. IgG (1 µg/IP) was used as negative IP control. QPCR was performed with primers located in the ZNF180 and L1TD1 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].

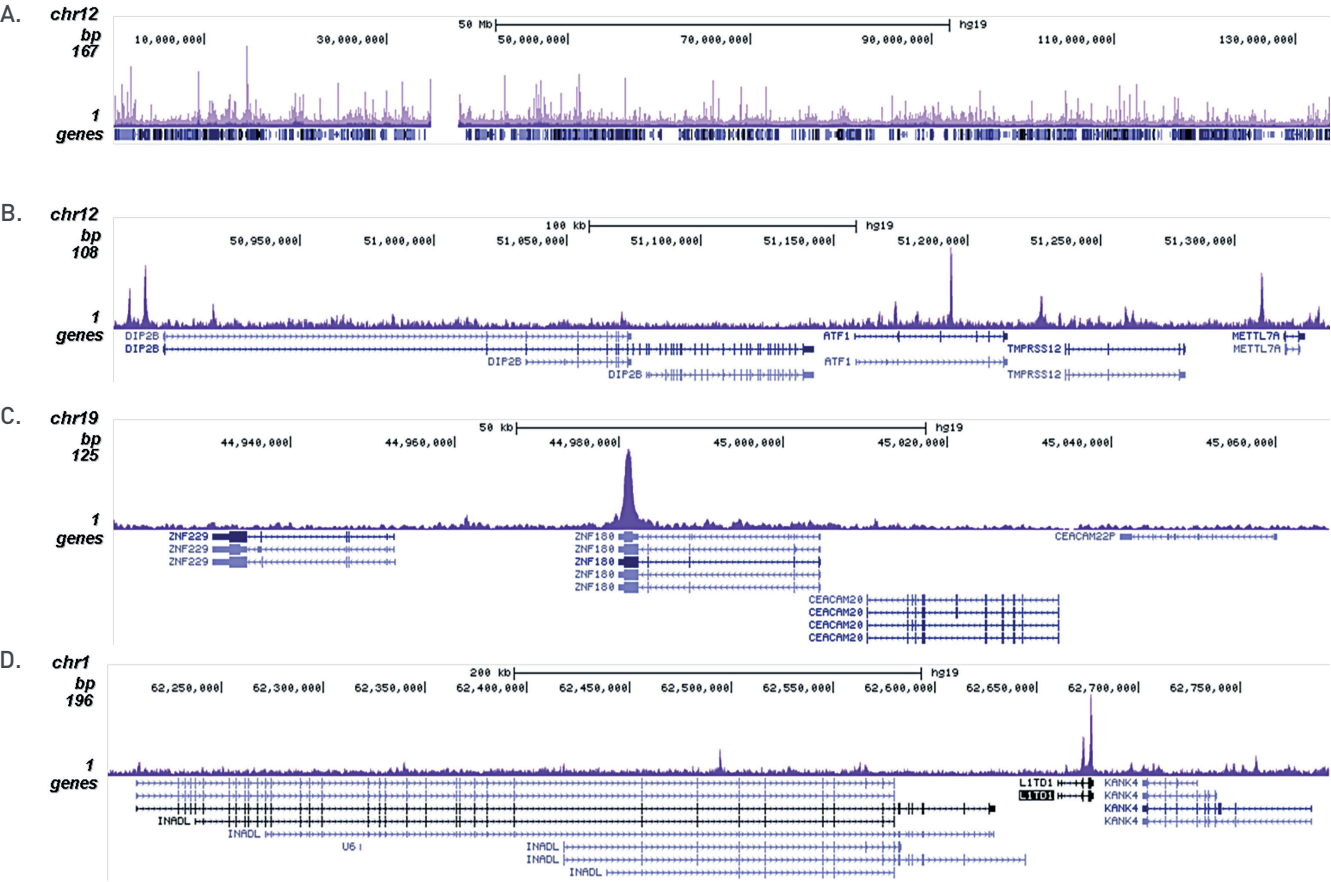


Figure 2. ChIP-seq results obtained with the Diagenode antibody directed against KAP1

ChIP was performed on sheared chromatin from 4 million HeLa cells using 2 µg of the Diagenode antibody against KAP1 (Cat. No. C15410236) 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 enrichment along the complete sequence and a 300 Kb region of human chromosome 12 (fig 2A and B), and in a two genomic regions surrounding the ZNF180 and L1TD1 positive control genes.

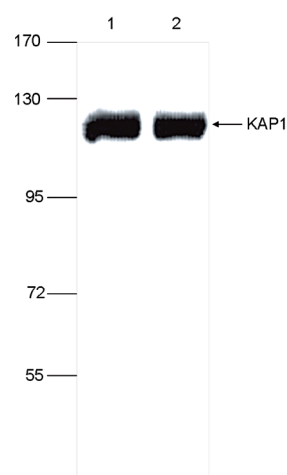


Figure 3. Western blot analysis using the Diagenode antibody directed against KAP1

Whole cell extracts (30 µg) from H1229 (lane 1) and HeLa cells (lane 2) were analysed by Western blot using the Diagenode antibody against KAP1 (Cat. No. C15410236) diluted 1:10,000. The position of the protein of interest is indicated on the right; the marker (in kDa) is shown on the left.

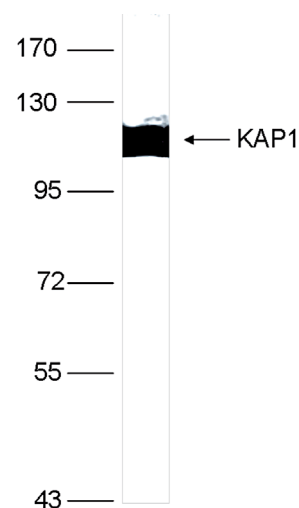


Figure 4. Western blot analysis using the Diagenode antibody directed against KAP1

Whole cell extracts (30 µg) from NIH3T3 cells were analysed by Western blot using the Diagenode antibody against KAP1 (Cat. No. C15410236) diluted 1:1,000. The position of the protein of interest is indicated on the right; the marker (in kDa) is shown on the left.

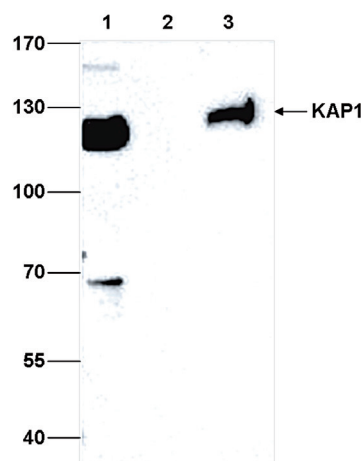


Figure 5. Immunoprecipitation using the Diagenode antibody directed against KAP1

Immunoprecipitation was performed on whole cell extracts from HeLa cells using 2 µg of the Diagenode antibody against KAP1 (Cat. No. C15410236). An equal amount of rabbit IgG was used as a negative control. The immunoprecipitated KAP1 protein was detected by western blot with the KAP1 antibody diluted 1:1,000. The IP with the KAP1 antibody and with the IgG negative control are shown in lane 3 and lane 2, respectively. Lane 1 shows the input (50 µg of HeLa whole cell extract).

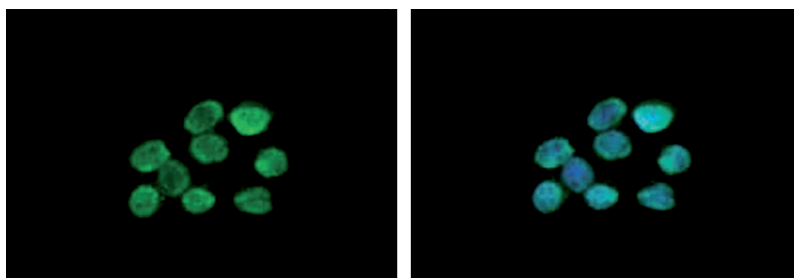


Figure 6. Immunofluorescence with the Diagenode antibody directed against KAP1

A431 cells were fixed with formaldehyde and stained with the Diagenode antibody against KAP1 (Cat. C15410236) diluted 1:200 (left). The right picture shows costaining with Hoechst 33342 nucleic acid stain.

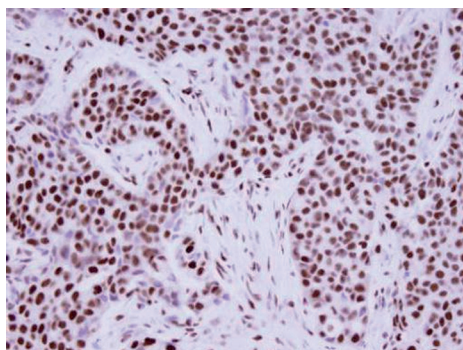


Figure 7. Immunohistochemistry using the Diagenode antibody directed against KAP1

Formalin fixed paraffin embedded breast carcinoma cells were stained with the Diagenode antibody against KAP1 (Cat. No. C15410236) diluted 1:500 followed by a peroxidase labelled goat anti-rabbit secondary antibody.

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Last update: March 4, 2015