diagenode
A Hologic Company

TECHNICAL DATASHEET

NFYB antibody

Cat. No. C15410241

Type: Polyclonal **ChIP/ChIP-seq grade**
Source: Rabbit
Lot: 42817
Size: 100 µg
Concentration: 0.42 µg/µl

Specificity: Human, mouse: positive
Other species: not tested.
Purity: Affinity purified polyclonal antibody
Storage: Store at -20°C; for long storage, store at -80°C. Avoid multiple freeze-thaw cycles.
Storage buffer: PBS containing 1% BSA, 20% glycerol and 0.025% proclin300.

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

Description: Polyclonal antibody raised in rabbit against NFYB (Nuclear transcription factor-Y, subunit B), using a recombinant protein.

Other names: NF-YB, HAP3, CBF-A, CBF-B

Applications

Applications	Suggested dilution	References
ChIP*	2 µg per ChIP	Fig 1, 2
Western blotting	1:500 - 1:3,000	Fig 3
Immunoprecipitation	2.5 µg per IP	Fig 4
Immunofluorescence	1:100 - 1:1,000	Fig 5

*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

NFYB (UniProt/Swiss-Prot entry P25208) is one of the 3 subunits of the ubiquitous transcription factor NFY. All three subunits A, B and C are required to form a NFY-DNA complex. There is a connection between mutant p53 gain of function, NF-Y transactivation and DNA damage.

Diagenode sa. BELGIUM | EUROPE
LIEGE SCIENCE PARK
Rue du Bois Saint-Jean, 3
4102 Seraing - Belgium
Tel: +32 4 364 20 50
Fax: +32 4 364 20 51
orders@diagenode.com
info@diagenode.com

Diagenode LLC. USA | NORTH AMERICA
400 Morris Avenue, Suite 101
Denville, NJ 07834 - USA
Tel: +1 862 209-4680
Fax: +1 862 209-4681
orders.na@diagenode.com
info.na@diagenode.com

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Results

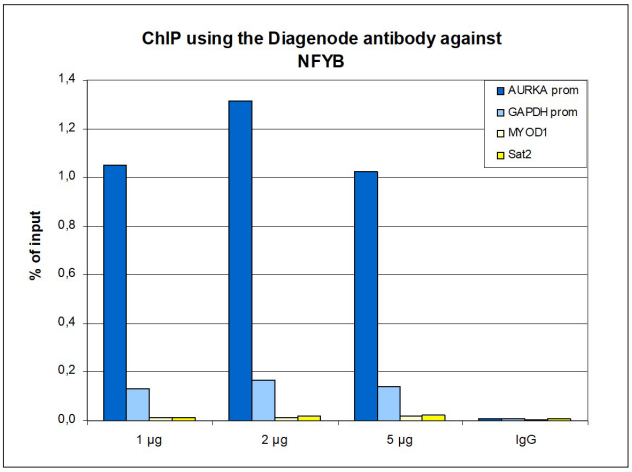

Figure 1. ChIP results obtained with the Diagenode antibody directed against NFYB

ChIP assays were performed using HeLa cells, the Diagenode antibody against NFYB (cat. No. C15410241) 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 for the AURKA and GAPDH promoters, 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).

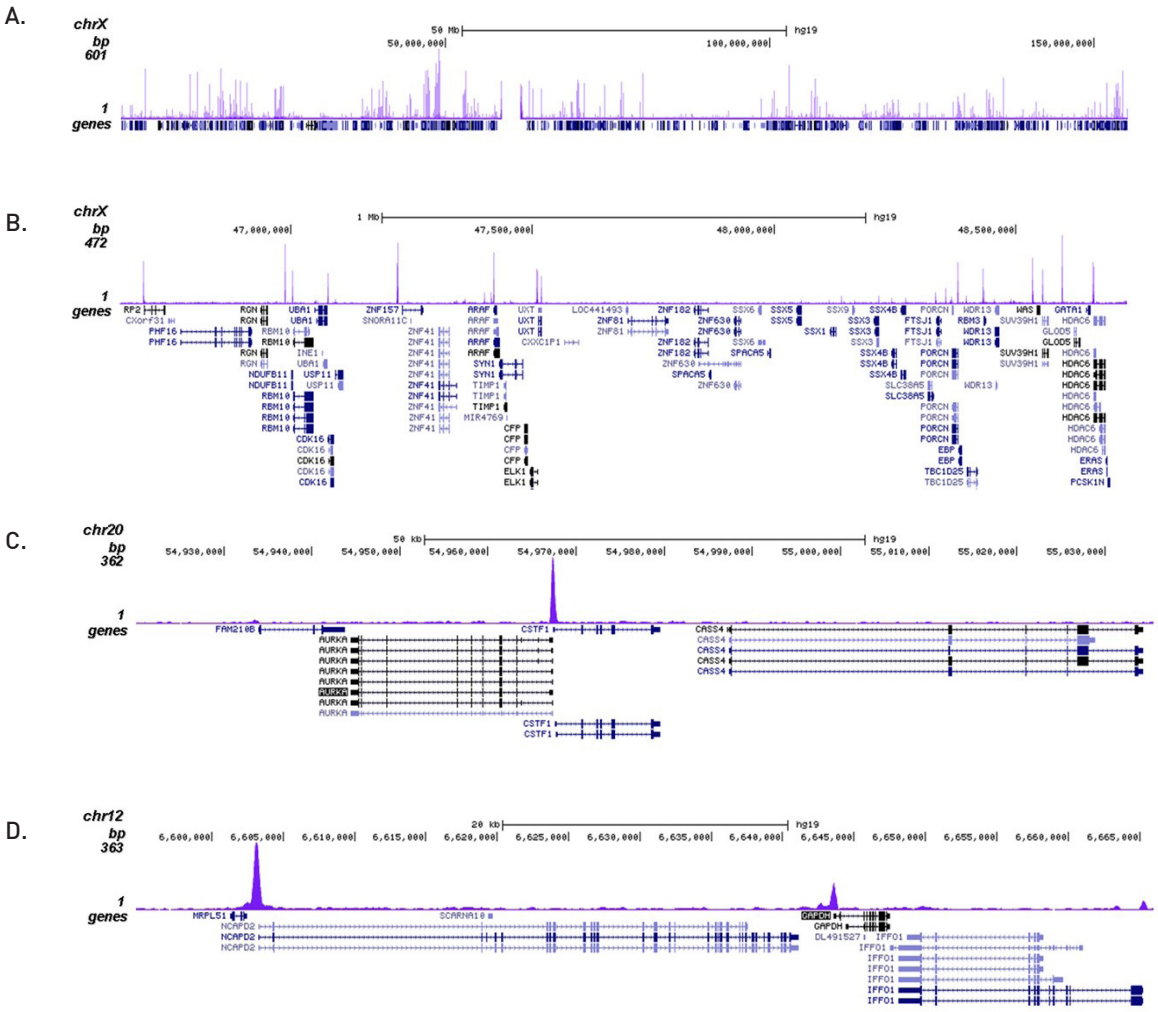

Figure 2. ChIP-seq results obtained with the Diagenode antibody directed against NFYB

ChIP was performed on sheared chromatin from 4 million HeLa cells using 2 µg of the Diagenode antibody against NFYB (cat. No. C15410241) 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 2 Mb region of the human X chromosome (fig 2A and B), and in two genomic regions surrounding the AURKA and GAPDH positive control genes.

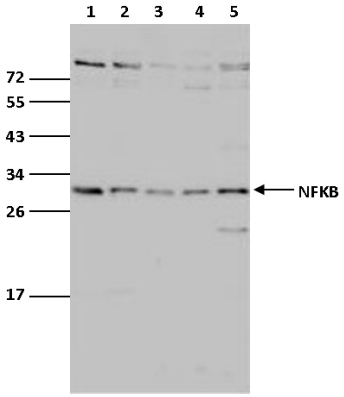

Figure 3. Western blot analysis using the Diagenode antibody directed against NFYB

Nuclear extracts from Neuro2A (lane 1), C8D30 (lane 2), NIH3T3 (lane 3), Raw264.7 (lane 4) and C2C12 (lane 5) cells (30 µg) were analysed by Western blot using the Diagenode antibody against NFYB (cat. No. C15410241) 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.

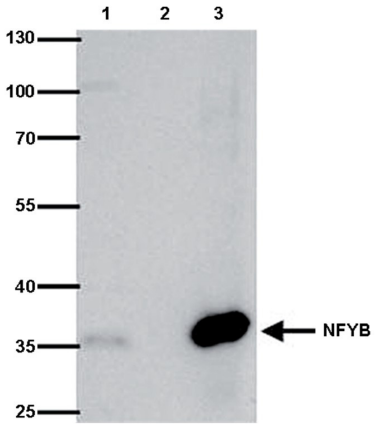

Figure 4. Immunoprecipitation using the Diagenode antibody directed against NFYB

Immunoprecipitation was performed on whole cell extracts from 293T cells using 2.5 µg of the Diagenode antibody against NFYB (cat. No. C15410241). An equal amount of rabbit IgG was used as a negative control. The immunoprecipitated NFYB protein was detected by western blot with the NFYB antibody diluted 1:1,000. The IP with the NFYB antibody and with the IgG negative control are shown in lane 3 and lane 2, respectively. Lane 1 shows the input (40 µg of 293T whole cell extract).

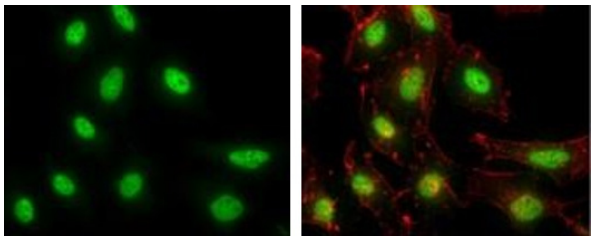

Figure 5. Immunofluorescence with the Diagenode antibody directed against NFYB

HeLa cells were fixed with formaldehyde and stained with the Diagenode antibody against NFYB (cat. C15410241) diluted 1:500 (left). The right picture shows costaining with the cytoskeleton marker Phalloidin.