



Technical Data Sheet

Diagenode sa
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Product name:
antibody directed against NF-YB
(Nuclear Factor-Y subunit B)

Catalog #: pAb-TFNFYB-100	Type: Polyclonal	Size: 100 µg/ 60 µl
Lot #: TF-0010	Source: Rabbit	Concentration: 1.7 µg/ µl

Description: This antibody has been raised against recombinant NF-YB protein.

Specificity: Human: positive
Other species: not tested

Applications	Suggested dilution	References
ELISA	Not tested	
Dot blotting	Not tested	
Western blotting	1:3000	
Gel Supershift	Not tested	
Immunocytochemistry	1:50 and 1:100	Ref 1., Fig 3
Flow cytometry	Not tested	
Immunoprecipitation	3 µg of ab/ 100 µg of nuclear extract	
ChIP	5 -10 µg per IP (3 -6 µl)	Fig 1., Fig 2

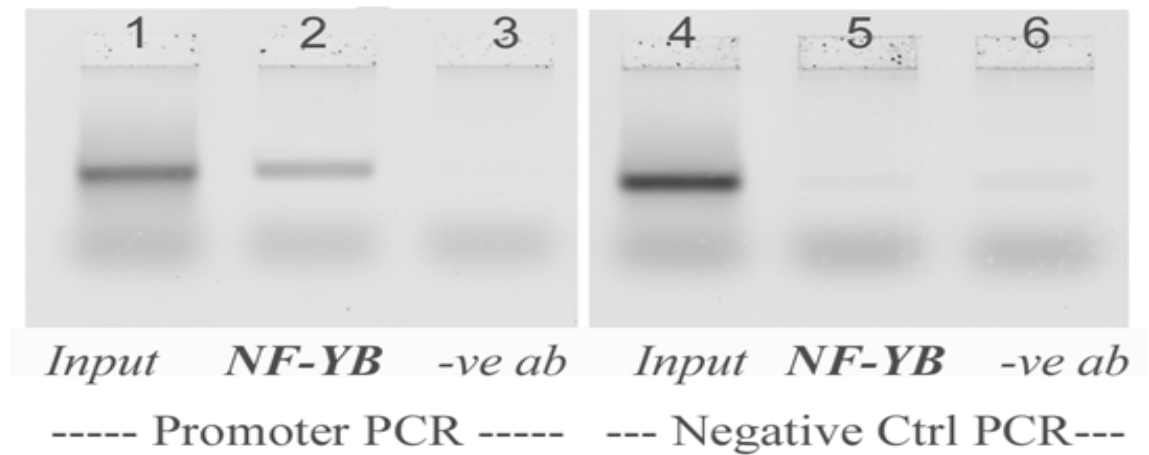
Format: In solution in PBS. The polyclonal antibody has been affinity purified.

Storage: Store at -20°C. Do not freeze-thaw.

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

Availability date: October 15, 2006
Last data sheet update: February 27, 2007

Figure 1

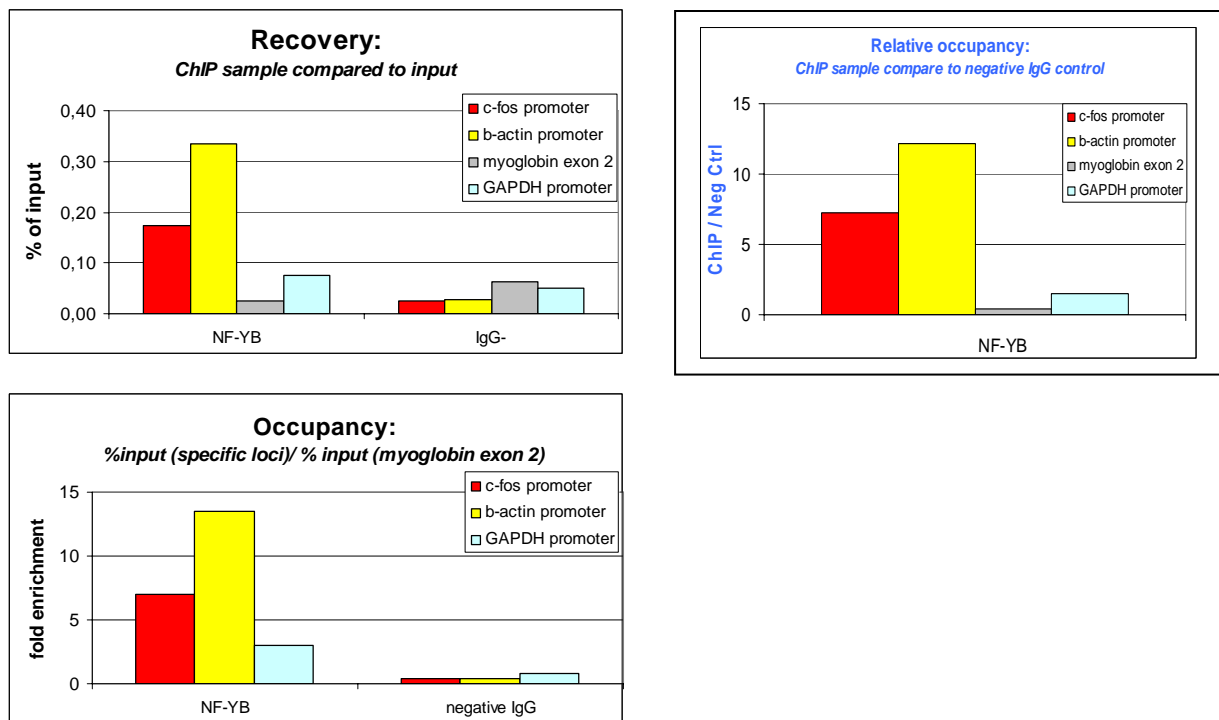


ChIP results obtained with the antibody anti-NF-YB from Diagenode.

ChIP assays were performed using HeLa cells, the Diagenode antibody directed against NF-YB and optimized PCR primer sets for end-point PCR. Chromatin sheared from 2×10^6 cells and $5 \mu\text{g}$ of antibody anti-NF-YB were used per ChIP experiment (lanes 2 and 5). A negative control antibody was included in the ChIP assay (lanes 3 and 6).

Occupancy of the Son promoter by NF-YB is evident based on the end-point PCR analysis of immunoprecipitated DNA (lane 2, positive sample). Controls for IP and PCR specificity include: *a/* PCR performed with the primers for the Sat. Cen11 centromeric region (lane 5, negative control); *b/* PCR was performed with each primer pairs and the input sample (as a positive control for input as well as primer pairs and PCR (lanes 1 and 4)); and *c/* IP was performed with a negative control antibody (lanes 3 and 6).

Figure 2



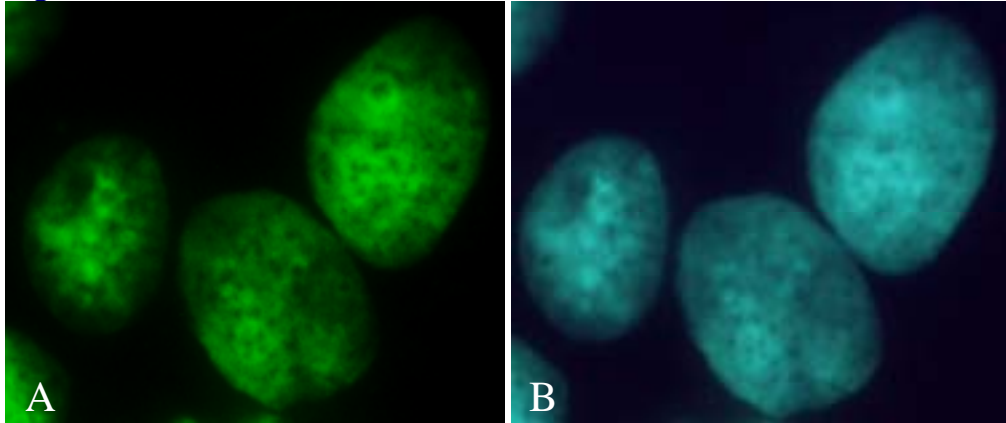
ChIP results obtained with the antibody anti-NF-YB from Diagenode.

ChIP assays were performed using HeLa cells, the Diagenode antibody directed against NF-YB and optimized PCR primer sets for qPCR. Two kits from Diagenode were also used: one to prepare the sheared chromatin ready-to-ChIP and the other to ChIP (respectively: the shearing module from the Red ChiP kit and the OneDay ChIP kit). Chromatin sheared from 2x 10e6 cells and 10 µg of antibody anti-NF-YB were used per ChIP experiment. A negative control antibody was included in the ChIP assay (negative IgG).

Left graphs: recovery and occupancy are shown. Right graph: relative occupancy is given.

Occupancy of the c-fos, b-actin and GAPDH promoters by NF-YB is evident based on the qPCR analysis of immunoprecipitated DNA. Controls for IP and PCR specificity include: *a/* PCR performed with the primers for myoglobin exon 2 (negative control); *b/* PCR was performed with each primer pairs and the input sample (as a positive control for input and positive control for the PCR and primer pairs); and *c/* IP was performed with a negative control antibody.

Figure 3



Immunohistochemistry results obtained with the antibody anti-NF-YB from Diagenode.

HeLa cells were stained with the antibody directed against NF-YB and with DAPI. Cells were methanol fixed for 10 minutes at -20°C and then blocked with 1% BSA containing PBS. The fixation step stabilises the morphology of the cells and permeabilizes membranes as well.

(A) Cells were immunofluorescently labeled with the Diagenode rabbit polyclonal antibody anti-NF-YB (diluted 1:100 and incubated for 1 hour at room temperature) followed by goat anti-rabbit antibody conjugated to FITC.

(B) Nuclei were DAPI stained to label specifically the DNA.

In both cases, the nuclei are clearly stained.

NF-YB quick overview

NFY is a ubiquitous transcription factor of 70 kDa resulting from the interaction of the three subunits A, B and C [2, 3, 4], all of which are required to form a NFY-DNA complex [5]. There is a connection between mutant p53 gain of function, NF-Y transactivation and DNA damage [6].

1. Frontini M., Imbriano C., Manni I. and Mantovani R. 2004 *Cell Cycle* 3(2):217-22.
2. Maity S.N., Sinha S., Ruteshouser E.C. and de Crombrughe B. 1992 *J Biol Chem* 267(23):16574-80.
3. Kim I.S., Sinha S., de Crombrughe B. and Maity S.N. 1996 *Mol Cell Biol* 16(8):4003-13.
4. Caretti G., Salsi V., Vecchi C., Imbriano C. and Mantovani R. 2003 *J Biol Chem.* 278(33):30435-40.
5. Sinha S., Maity S.N., Lu J. and de Crombrughe B. 1995 *Proc Natl Acad Sci U S A* 92(5):1624-8.
6. Peart M.J. and Prives C. 2006 *Cancer Cell* 10(3):173-4