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Datasheet for 200-401-100

NF-Y Antibody

Overview

Description:	Anti-NF-Y (A subunit) (RABBIT) Antibody - 200-401-100
Item No.:	200-401-100
Size:	100 µg
Applications:	WB, ChIP, EMSA, IF, IHC
Reactivity:	Human, Monkey
Host Species:	Rabbit

Product Details

Background:	Anti-NF-Y antibody detects NF-Y (a subunit) protein. NF-Y is a highly conserved transcription factor that stimulates the transcription of various genes by recognizing and binding to a CCAAT motif in promoters, for example in type 1 collagen, albumin and beta-actin genes. NF-subunit A associates with a tight dimer composed of the NF-YB and NF-YC subunits, resulting in a trimer that binds to DNA with high specificity and affinity. Anti-NF-YA Antibody is ideal for investigators involved in Cell Signaling, Immunology, Cancer, and Signal Transduction research.
Synonyms:	rabbit anti-NF-Y Antibody, Nf-Y-A subunit, CAAT box DNA-binding protein subunit A, Nuclear transcription factor Y subunit A
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	IgG

Target Details

Gene Name:	NFYA
Reactivity:	Human, Monkey
Immunogen Type:	Conjugated Peptide
Immunogen:	Anti-NF-Y (A) antibody was produced by repeated immunizations with a synthetic NF-Y (A subunit) peptide corresponding to a region near the N-terminus of the human protein conjugated to Keyhole Limpet Hemocyanin (KLH).

Purity/Specificity:	Anti-NF-Y (a) antibody was prepared from monospecific antiserum by a multi-step procedure that includes delipidation, salt fractionation and ion exchange chromatography. A single precipitin arc was observed against anti-Rabbit Serum when assayed by immunoelectrophoresis.
Relevant Links:	• NCBI - CAI20286.1 • UniProtKB - P23511 • GeneID - 4800

Application Details

Tested Applications:	WB
Suggested Applications:	ChIP, EMSA, IF, IHC (Based on references)
Application Note:	Anti-NF-Y antibody is tested for western blotting and suitable for ELISA. Researchers should determine optimal titers for applications that are not stated below.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:10,000
WB:	1:1,000

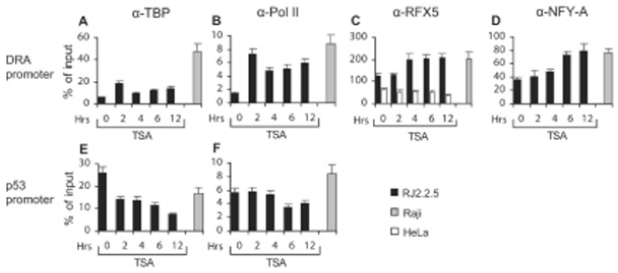
Formulation

Physical State:	Liquid (sterile filtered)
Concentration:	0.60 mg/mL by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	None

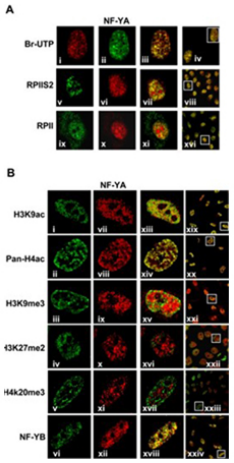
Shipping & Handling

Shipping Condition:	Dry Ice
Storage Condition:	Store vial at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.
Expiration:	Expiration date is one (1) year from date of receipt.

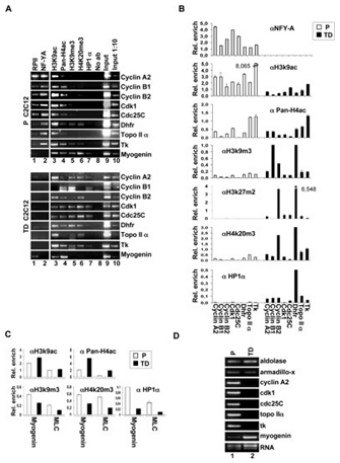
Images



ChIP
Enhanced recruitment of enhanceosome and general transcription components correlate with responsiveness to TSA. ChIP with antibodies against TBP (A and E) and the large subunit of Pol II (B and F) were amplified by real-time PCR with DRA (A and B,) and p53 (E and F) primers from the indicated cells and time points as in Figure 3. The RFX5 in RJ2.2.5 and HeLa (C) and NFY-A (D) enhanceosome components monitored on the DRA promoter during the TSA time course.
Fig 4.
PMID: 16452299

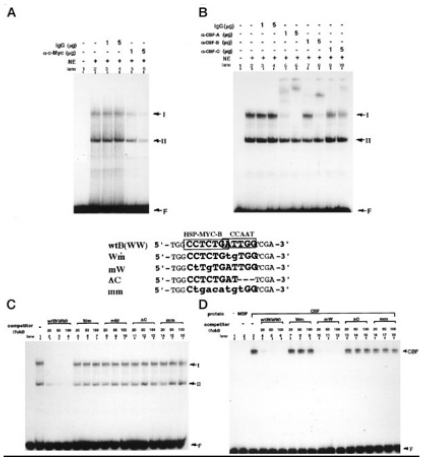


Immunofluorescence Microscopy
(A) NF-Y is associated with active transcription sites in living cells. After in vivo incorporation of BrUTP (run-on), cells were fixed and endogenous NF-YA (ii) and nascent RNA transcripts (i) were detected by indirect immunofluorescence combined with Confocal Scanning Laser Microscopy by using anti-NF-YA and anti-BrU antibodies. In the overlay (iii), yellow indicates colocalizations between NF-YA (green) and transcription sites (red). In panels vi and x cells were immunostained with anti-NF-YA, in panels v and ix with anti-RPII CTD repeat YSPTSPS (phospho S2) and anti-total RPII respectively. The majority of NF-YA (red) colocalizes with the activated form of RPII (green)(vii). (B) Cells were immunostained with anti-NF-YA (vii-xii), -acetylated H3K9 (i), -acetylated H4 (ii), -tri-methylated H3K9 (iii), -di-methylated H3K27 (iv), -tri-methylated H4K20 (v) and -NF-YB (vi) antibodies. The majority of NF-YA colocalizes with acetylated (xiii, xiv), but poor colocalization occurs with methylated histones (xv, xvi,xvii). Panel xviii shows the overlay of two subunits of NF-Y, NF-YA (xii) and NF-YB (vi). Panels from xix to xxiv represent a typical optical field of the merge. In figure 1A and 1B confocal analysis of single optical section is shown. The images have been collected with a 60x objective.
Figure 1. PMID: 18431504



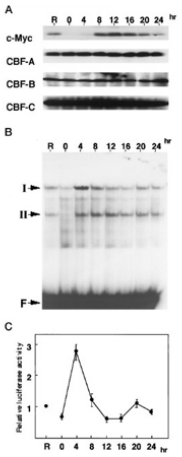
Western Blot

Histone acetylation correlates with NF-Y recruitment onto its target promoters.
(A) ChIPs performed on proliferating (P) and terminally differentiated (TD) C2C12 cells using the indicated antibodies. No antibody was used as control (No Ab). (B) Q-ChIP analysis on proliferating (P) and terminally differentiated (TD) primary myoblasts, performed with the indicated antibodies. The promoters analyzed were:
1) CyclinA2, 2) CyclinB1, 3) CyclinB2, 4) Cdk1,
5) Cdc25C, 6) Dhfr, 7) TopoisomeraseIIα, 8) Tk. (C) Q-ChIP analysis of the Myogenin and MLC promoters performed with the indicated antibodies on proliferating (P) and terminally differentiated (TD) primary myoblasts. On B and C the enrichment of immunoprecipitated promoter fragments relative to the input was done. Rabbit IgG was used as control. (D) RT-PCR amplification of indicated genes was performed on proliferating (P) and terminal differentiated (TD) C2C12. Figure 2. PMID: 18431504



Western Blot

Nucleoprotein complex formation on the wtB sequence. Bandshift assays were carried out using the Balb 3T3 nuclear extract (NE) and the labeled wtB sequence as a probe. Prior to the addition of the probe, the reaction mixture was incubated for 60 min at 0 °C in the presence or absence of the indicated amounts of antibodies against c-Myc (OM11–905) (A) or three CBF/NF-Y subunits (B) (pRαYB, pRαYA/C, or α-CBF-C), or of nonspecific IgG. The labeled probe was then added to the mixture, and the mixture was further incubated at room temperature for 30 min and analyzed in a 4% polyacrylamide gel. Similar bandshift assays on the wtB sequence were carried out using either the Balb 3T3 nuclear extract (C) or purified CBF subunits as MBP fusion proteins (D) in the presence of non-labeled oligonucleotides. Increasing amounts (× 20, 50, and 100 of the probe) of non-labeled oligonucleotides corresponding to the wild type (WW) or various mutants (Wm, mW, mm, and ΔC) of the wtB sequence (see middle panel) were added to the reaction mixtures as competitors. The positions of two specific DNA-protein complexes are indicated
by arrows (I and II). F shows the position of free probes.
Figure 4. PMID: 10446203



Western Blot
Changes in the CBF·NF-Y complex formation and the transcriptional activity of the wtB sequence during the cell cycle. Mouse Balb 3T3 cells were synchronized in the G0 phase of the cell cycle by serum starvation for 48 h. The nuclear extracts were prepared from the cells harvested at various times (0–24 h) after serum addition and examined for the expression of c-Myc and the CBF subunits (A) and the complex formation on the wtB sequence (B). R indicates the samples from the cells at random culture. A, the extracts were Western blotted using the anti-c-Myc antibody (C-33) or the antibody against the subunits of CBF/NF-Y (pRαYB, pRαYA/C, or α-CBF-C). B, the extracts were subjected to bandshift assays using a labeled wtB sequence as a probe. The positions of the specific complex I, II, and free probes are indicated. C, 2 μg of the reporter plasmid pwtB-TATA-Luc was transfected to Balb 3T3 cells, and the cells were synchronized as described previously (12). The luciferase activity was assayed at various times (0–24 h) after serum addition. Relative luciferase activities to that of the cells at random culture (R) are shown.
Figure 8. PMID: 10446203



Western Blot
Western Blot of Rabbit Anti-NF-Y(A subunit) Antibody. Lane 1: CHO-7 cells in the absence (I) of cholesterol. Lane 2: CHO-7 cells in the presence (S) of cholesterol. Load: Equivalent aliquots of chromatin from each sample containing ~50 μg of total protein per lane. Primary antibody: anti-NF-YA antibody at 1:1000 for overnight at 4°C. Secondary antibody: HRP conjugated Goat-anti-Rabbit IgG [H&L] secondary antibody (p/n 611-103-122) at 1:10,000 for 45 min at RT. Block: 5% BLOTTO overnight at 4°C. Predicted/Observed size: doublet of ~ 42 kDa for NF-Y A.

References

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