Product Datasheet

c-Myc Antibody - BSA Free
NB600-336

Unit Size: 0.1 ml

Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

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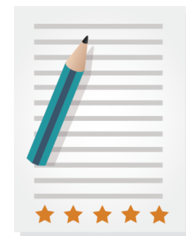


NB600-336

c-Myc Antibody - BSA Free

Product Information

Unit Size	0.1 ml
Concentration	1 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.09% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	Tris-Glycine, 0.15 M NaCl
Target Molecular Weight	48.8 kDa

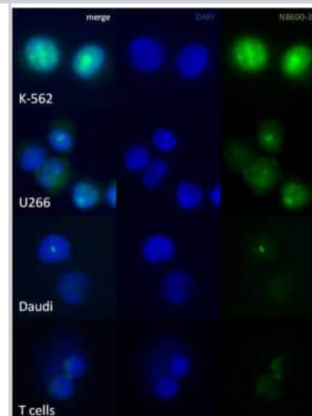
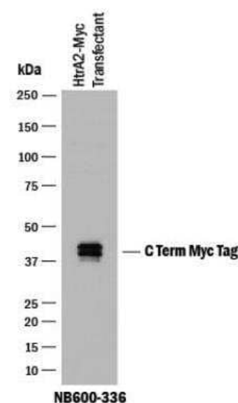
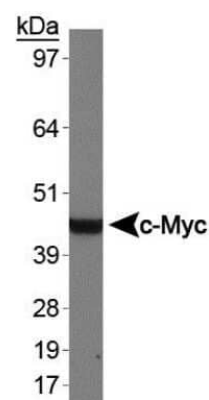
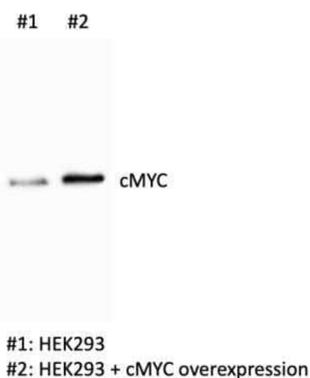
Product Description

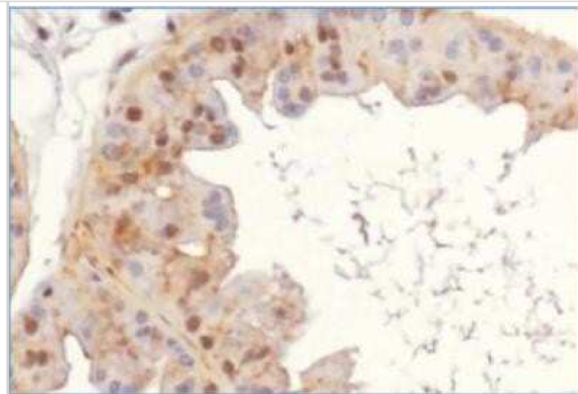
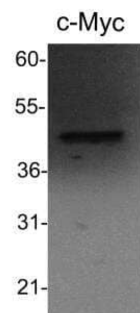
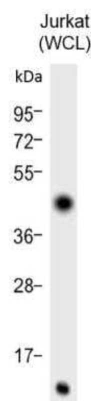
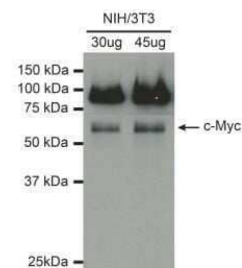
Description	Novus Biologicals Rabbit c-Myc Antibody - BSA Free (NB600-336) is a polyclonal antibody validated for use in IHC, WB, ELISA, ICC/IF, Simple Western, IP and ChIP. Anti-c-Myc Antibody: Cited in 27 publications. All Novus Biologicals antibodies are covered by our 100% guarantee.
Host	Rabbit
Gene ID	4609
Gene Symbol	MYC
Species	Human, Mouse, Insect, S. pombe
Reactivity Notes	Insect reactivity reported in scientific literature (Martinez-Velez N et al). Use in S. pombe reported in scientific literature (PMID:18094683).
Immunogen	A synthetic peptide made to the human c-Myc Antibody (between residues 385-435) [Uniprot: P01106]

Product Application Details

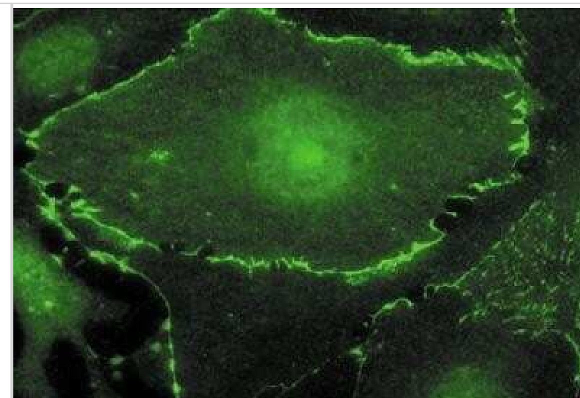
Applications	Western Blot, Simple Western, Immunohistochemistry-Paraffin, ELISA, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunoprecipitation, Chromatin Immunoprecipitation (ChIP)
Recommended Dilutions	Western Blot 1:1000, Simple Western 1:500, ELISA 1:1000, Immunohistochemistry 1:100, Immunocytochemistry/ Immunofluorescence 1:50, Immunoprecipitation 1:1000, Immunohistochemistry-Paraffin 1:100, Chromatin Immunoprecipitation (ChIP)
Application Notes	Prior to immunostaining paraffin tissues, antigen retrieval with sodium citrate buffer (pH 6.0) is recommended. In Simple Western only 10 - 15 uL of the recommended dilution is used per data point. See Simple Western Antibody Database for Simple Western validation: Tested in Jurkat lysate 0.05 mg/mL, separated by Size, antibody dilution of 1:500, apparent MW was 87 kDa. Separated by Size-Wes, Sally Sue/Peggy Sue.

Images

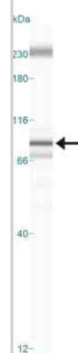
Immunocytochemistry/Immunofluorescence: c-Myc Antibody [NB600-336] - Staining of K-562, U266, Daudi and normal T cells with c-Myc antibody. ICC/IF image submitted by a verified customer review.	
Western Blot: c-Myc Antibody [NB600-336] - Detection of c-Myc in HtrA2 -Myc Transfectant lysates .	
Western Blot: c-Myc Antibody [NB600-336] - Analysis of c-Myc on Jurkat whole cell extract using NB600-336.	
Western Blot: c-Myc Antibody [NB600-336] - Lane 1: control HEK293 cell lysate. Lane 2: CMYC over-expression in HEK293 cell lysate. WB image submitted by a verified customer review.	

Immunohistochemistry: c-Myc Antibody [NB600-336] - Analysis of c-Myc in mouse prostate using DAB with hematoxylin counterstain.	
Western Blot: c-Myc Antibody [NB600-336] - c-Myc in MOLT-4 cells. WB image submitted by a verified customer review.	
Western Blot: c-Myc Antibody [NB600-336] - Analysis of whole cell lysate (WCL) of Jurkat cells using anti-cMyc antibody (NB600-336) at 1:1000 dilution. HRP conjugated goat anti-rabbit IgG (H+L) cross adsorbed secondary antibody was used with ECL substrate for the detection of c-Myc antibody bound to the blotted protein. This c-Myc antibody detected the c-Myc specific band at its expected position (48-50kDa). The signal below 10 kDa in this blot is potentially the degraded protein and we have not characterized this band.	
Western Blot: c-Myc Antibody [NB600-336] - Analysis using the HRP conjugate of NB600-336. Detection of c-Myc in NIH/3T3 cell lysates (30ug and 45ug per lane) using anti-c-Myc antibody. WB image submitted by a verified customer review.	

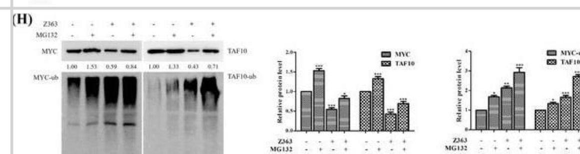
Immunocytochemistry/Immunofluorescence: c-Myc Antibody [NB600-336] - Detection of c-myc Tagged Plakoglobin by Immunofluorescence. Samples: Human microvascular endothelial cells expressing c-myc tagged plakoglobin following transient transfection.



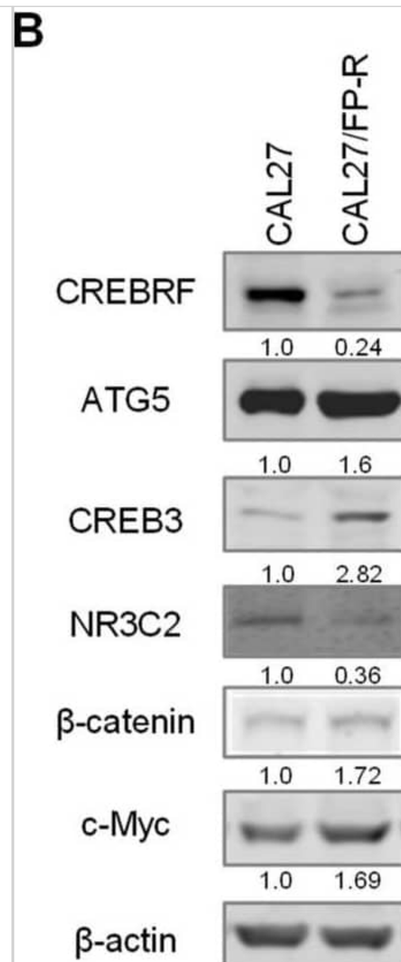
Simple Western: c-Myc Antibody [NB600-336] - Lane view shows a specific band for c-Myc in 0.05 mg/ml of Jurkat lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.



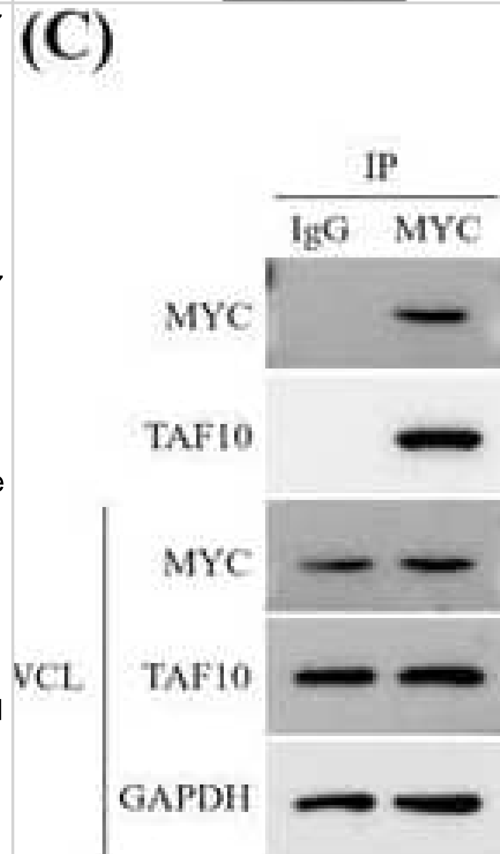
Z363 promotes MYC and TAF10 degradation. (A) Identification of small inhibitory molecules for MYC. (B) MCF7 cells were treated with Z363 (0, 2.5, 7.5 and 15 µg/ml) for 24 h. The protein levels of MYC and TAF10 were analysed by Western blotting. (C) MCF7 cells were treated with Z363 (7.5 µg/ml) for 0, 6, 12 and 24 h. Furthermore, the protein levels of MYC and TAF10 were analysed by Western blotting. (D) MCF7 cells were treated with 25 µM MG132 at the indicated time points, followed by treatment with or without Z363 (7.5 µg/ml) for 24 h, and MYC and TAF10 expressions were analysed by Western blotting. (E) Western blots for MYC, phosphorylated MYC T58 and S62 in MCF7 cells treated with Z363 at the times indicated. (F) Ratios of pT58 or pS62 to total MYC protein levels from the experiment (E). (G) IF staining for Ki67 and pT58 in Z363‐treated MCF7 cells, scale bar, 10 µm. (H) MCF7 cells were treated with 25 µM MG132 for 2 h, followed by Z363 treatment (7.5 µg/ml) for 24 h. Expressions of MYC and TAF10 were assessed using Western blot analysis. Data shown in F were analysed by two‐way ANOVA. Fluorescence images and blots were representative of three independent experiments. All data are presented as the mean +/- SEM of n = 3. ***p < .001, ns, no significance Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/36639831), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



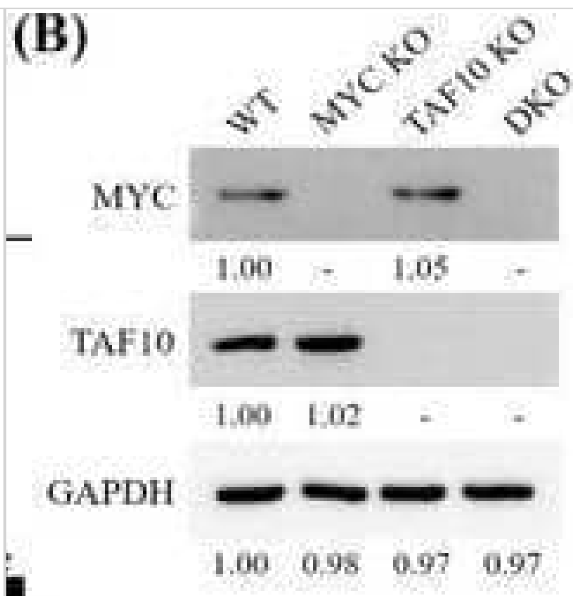
The role of miR124-3p and miR766-3p target genes in HNSCC drug resistance. (A) Expression analysis of miR124-3p and miR766-3p direct target genes and downstream target genes by Western blot in HNSCC cell lines (CAL27 and FaDu), with or without transfection with miRNA inhibitors or miRNA mimics. Left: Western blotting showed the expression of miR124-3p target gene (CREBRF) and CREBRF target genes (ATG5 and CREB3). Right: Western blotting showed the expression of miR766-3p target gene (NR3C2) and NR3C2 target genes (β-catenin and c-Myc). Quantitative data (relative expression levels after β-actin-corrected) from three independent experiments are disclosed below each protein band. Data represent the mean +/- SD (n = 3). (B) Target gene analysis in sensitive (CAL27) vs. resistant (CAL27/FP-R) HNSCC cell lines. Quantitative data (relative expression levels after β-actin-corrected) is shown below each protein band. (C) The effect of NR3C2 and/or CREBRF knockdown on drug-induced cytotoxicity in CAL27 and FaDu. Cells were transfected by 10 nM siRNA (single or combined) for 24 h followed by 72 h exposure to the indicated drug. Cytotoxicity was determined by MTT assay. IC50 values are listed in Table S7. (D) Measurement of apoptosis in CAL27 or FaDu cells in response to cisplatin or 5-FU +/- 24 h prior transfection with 10 nM siRNA. After 24 h of drug treatments, cells were labeled with anti-annexin V-FITC antibody and PI and then analyzed with flow cytometry. A two-way ANOVA with Bonferroni's correction for multiple comparisons was used to analyze group comparisons, and data are presented as means +/- SD (n = 3). * $p < 0.05$, *** $p < 0.001$ (vs. control siRNA), and ### $p < 0.001$ (vs. untreated). Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/36358691), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



TAF10 promotes cancer cell proliferation and migration. (A and B) MCF7 cells were transfected with Flag□labelled MYC and HA□labelled TAF10, and the interaction between MYC and TAF10 was detected by Co□IP. (C) Endogenous interaction of MYC and TAF10 was tested in MCF7 cells. (D) Schematic representation of MYC mutants. (E) Schematic representation of TAF10 mutants. Co□IP was used to detect the interaction between the TAF10 mutants and MYC in MCF7 cells. Co□IP was used to detect the interaction. (G) Interaction between the MYC mutants and TAF10 in MCF7 cells was detected using Co□IP. (H) MCF7 cells were co□transfected with empty vector (Vec) and HA□labelled TAF10 wild□type (HA□TAF10 WT) or mutants (HA□TAF10 Δ1□116, HA□TAF10 Δ116□206, HA□TAF10 Δ206□218). The MYC promoter activity was analysed using the dual luciferase reporter assay 24 h later. (I) Overexpression of TAF10 assessed the capacity for colony formation. MCF7 cells were transfected with either Control□vec or HA□TAF10. The ability of cells to form colonies was measured using crystal violet staining. (J) Overexpression of TAF10 measured the migration capacity of cells. MCF7 cells were transfected with either Control□vec or HA□TAF10. Transwell assays were used to assess the migration capacity of cells. Scale bar, 20 μm. Data shown in H were analysed by one□way ANOVA. Data shown in I and J were analysed by t□test. The blots represented three independent experiments. All data are presented as the mean +/- SEM of n = 3. ***p < .001, **p < .01, ns, no significance Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/36639831), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Co□inhibition of MYC and TAF10 causes synergistic reduction of cell proliferation and tumour growth. (A) Cells (WT, TAF10 KO) were treated with different doses of Z363 for 24 h. Cell proliferation was determined by the CCK□8 assay. (B) Knockout reliability was detected by Western blotting. (C) The proliferation of wild□type cells (WT), single and double KO cells (MYC KO, TAF10 KO, DKO) and Z363□treated cells were detected by Ki67 ELISA kit. (D) The apoptosis of wild□type cells (WT), single and double KO cells (MYC KO, TAF10 KO, DKO) and Z363□treated cells were detected by flow cytometry. (E) Cell apoptosis□related proteins levels were detected by Western blotting. (F) The migration of wild□type cells (WT), single and double KO cells (MYC KO, TAF10 KO, DKO), and Z363□treated cells were detected by a Transwell migration assay. Scale bar, 20 μm. (G) Representative images showing xenograft tumours at day 28 post□subcutaneous injection (n = 5). (H and I) Tumours were measured and depicted as tumour volume (H) or tumour weight (I). Data shown in A were analysed by two□way ANOVA. Data shown in C, D, F, H and I were analysed by one□way ANOVA. Flow cytometry, transwell and blots were representative of three independent experiments. All data are presented as the mean +/- SEM of n = 3. ***p < .001, **p < .01, *p < .05 Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/36639831), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



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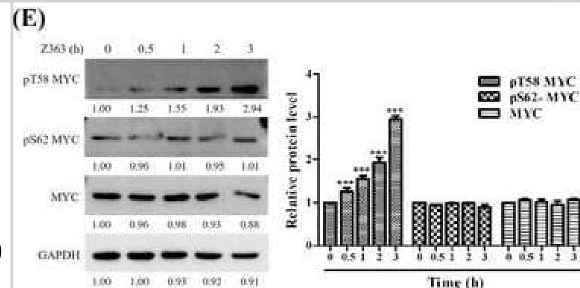
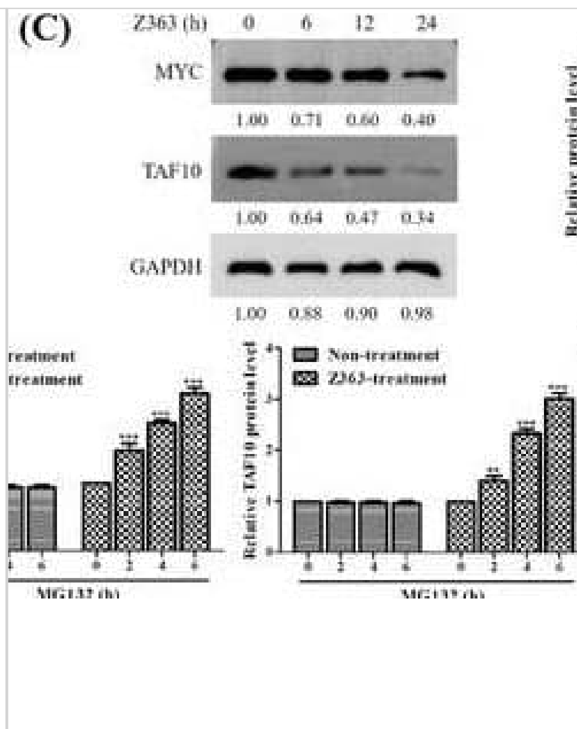
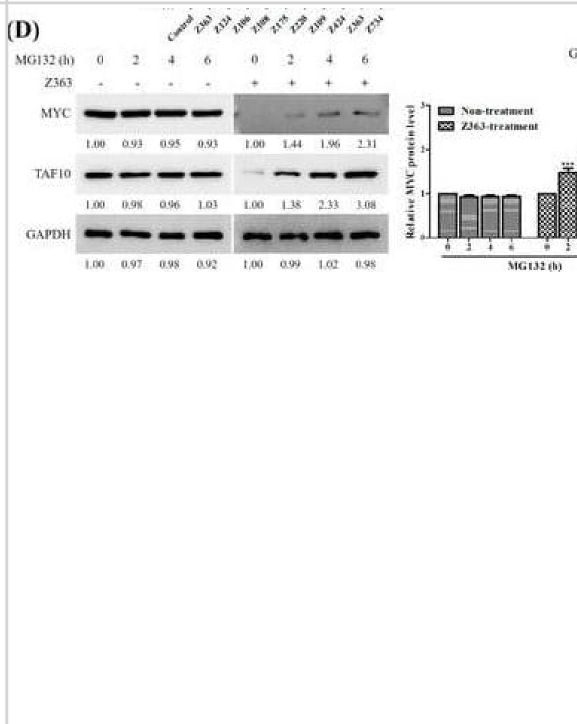


Z363 promotes MYC and TAF10 degradation. (A) Identification of small inhibitory molecules for MYC. (B) MCF7 cells were treated with Z363 (0, 2.5, 7.5 and 15 µg/ml) for 24 h. The protein levels of MYC and TAF10 were analysed by Western blotting. (C) MCF7 cells were treated with Z363 (7.5 µg/ml) for 0, 6, 12 and 24 h. Furthermore, the protein levels of MYC and TAF10 were analysed by Western blotting. (D) MCF7 cells were treated with 25 µM MG132 at the indicated time points, followed by treatment with or without Z363 (7.5 µg/ml) for 24 h, and MYC and TAF10 expressions were analysed by Western blotting. (E) Western blots for MYC, phosphorylated MYC T58 and S62 in MCF7 cells treated with Z363 at the times indicated. (F) Ratios of pT58 or pS62 to total MYC protein levels from the experiment (E). (G) IF staining for Ki67 and pT58 in Z363□treated MCF7 cells, scale bar, 10 µm. (H) MCF7 cells were treated with 25 µM MG132 for 2 h, followed by Z363 treatment (7.5 µg/ml) for 24 h. Expressions of MYC and TAF10 were assessed using Western blot analysis. Data shown in F were analysed by two□way ANOVA. Fluorescence images and blots were representative of three independent experiments. All data are presented as the mean +/- SEM of n = 3. ***p < .001, ns, no significance Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/36639831), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	
Z363 promotes MYC and TAF10 degradation. (A) Identification of small inhibitory molecules for MYC. (B) MCF7 cells were treated with Z363 (0, 2.5, 7.5 and 15 µg/ml) for 24 h. The protein levels of MYC and TAF10 were analysed by Western blotting. (C) MCF7 cells were treated with Z363 (7.5 µg/ml) for 0, 6, 12 and 24 h. Furthermore, the protein levels of MYC and TAF10 were analysed by Western blotting. (D) MCF7 cells were treated with 25 µM MG132 at the indicated time points, followed by treatment with or without Z363 (7.5 µg/ml) for 24 h, and MYC and TAF10 expressions were analysed by Western blotting. (E) Western blots for MYC, phosphorylated MYC T58 and S62 in MCF7 cells treated with Z363 at the times indicated. (F) Ratios of pT58 or pS62 to total MYC protein levels from the experiment (E). (G) IF staining for Ki67 and pT58 in Z363□treated MCF7 cells, scale bar, 10 µm. (H) MCF7 cells were treated with 25 µM MG132 for 2 h, followed by Z363 treatment (7.5 µg/ml) for 24 h. Expressions of MYC and TAF10 were assessed using Western blot analysis. Data shown in F were analysed by two□way ANOVA. Fluorescence images and blots were representative of three independent experiments. All data are presented as the mean +/- SEM of n = 3. ***p < .001, ns, no significance Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/36639831), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	

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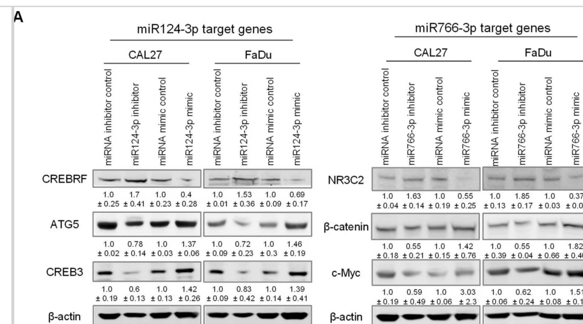
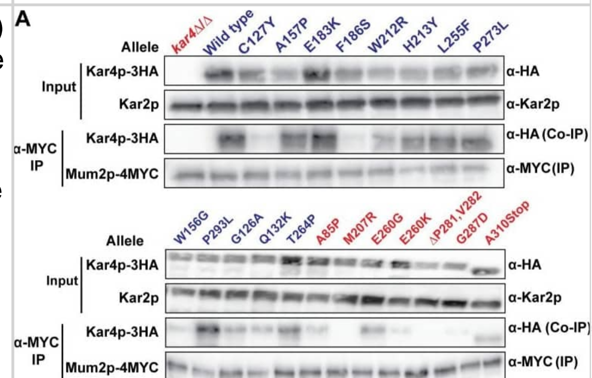
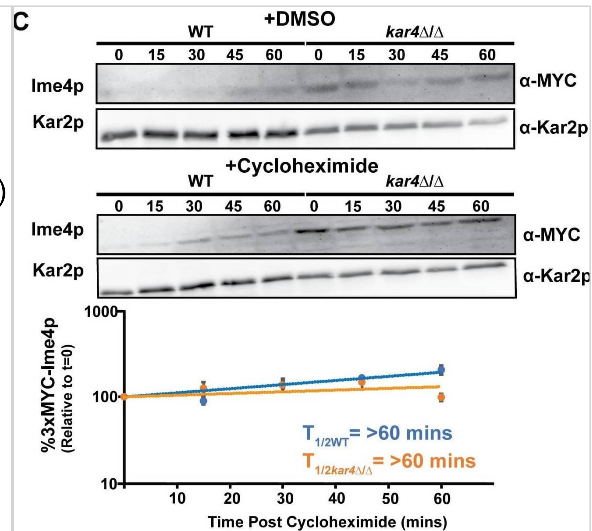


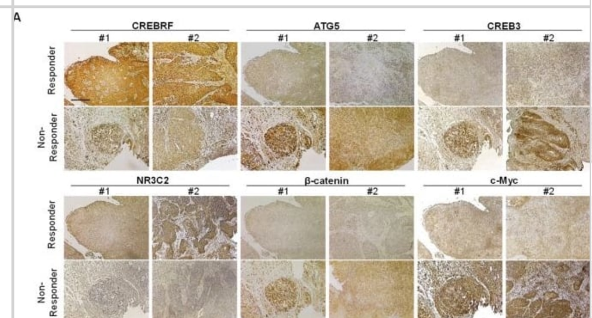
Kar4p interacts with the MIS complex components Mum2p and Slz1p.(A) Western blots of total protein and co-IPs between Mum2p-4MYC and the alleles of Kar4p-3HA. (MY 16256 and 16257) (Top) Total protein samples from the extracts that were used for the Co-IPs. Kar2p is used as a loading control. Alleles proficient for Kar4p's meiotic function (Mei+) are in blue and alleles in red are defective (Mei-). (Bottom) Co-IPs where Mum2p-4MYC was purified and the co-purification of Kar4p-3HA was assayed. (B) Western blots of total protein and Co-IPs between Slz1p-3HA and Kar4p-9MYC. (MY 16405 and 16409) (Left) Total protein samples from the extracts that were used for the co-IPs. "*" indicates a non-specific band. (Right) Co-IPs where Slz1p-3HA was purified and the co-purification of Kar4p-13MYC was assayed. "‡" indicates the heavy chain of IgG from the anti-HA magnetic beads used for the Co-IP. (C) Western blots of total protein and Co-IPs between Kar4p-9MYC and Slz1p-3HA. (Left) Total protein samples from the extracts that were used for the Co-IPs. (Right) Co-IPs where Kar4p-9MYC was purified and the co-purification of Slz1-3HA was assayed. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/37603553), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



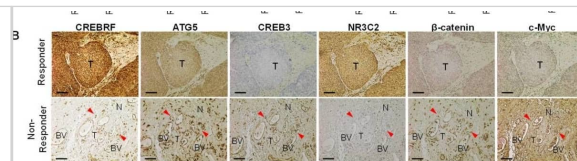
Kar4p is required for mRNA m6A methylation.(A) mRNA m6A levels measured using an ELISA-like assay. The indicated mutations were made in the SK1 strain background (MY 16325, 16326, 16351, 16353, and 16356) and samples were harvested after four hours of exposure to meiosis inducing conditions. Experiments were run in three biological replicates for each strain and error bars represent standard deviation. (B) Western Blot of 3xFLAG-Rme1p in wild type (MY 16563) and kar4Δ/Δ (MY 16569) across a time course of meiosis. Kar2p is used as a loading control. (C) Western blot of 3xMYC-Ime4p after four hours in meiosis inducing media with either 100 μM cycloheximide or an equivalent amount of DMSO in both wild type (Say914) and kar4Δ/Δ (MY 16543). (Top) 3xMYC-Ime4p levels with DMSO. (Middle) 3xMYC-Ime4p levels with cycloheximide. (Bottom) Quantification of three biological replicates of the cycloheximide chase experiment. The strains used are in the SK1 background. Kar2p is used as a loading control. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/37603553), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Downregulation of CREBRF and NR3C2 increase poor prognosis in HNSCC. (A) Histological analysis of miR124-3p and miR766-3p target gene expression (CREBRF-ATG5/CREB3, NR3C2-β-catenin/c-Myc) in Responder vs. Non-Responder HNSCC clinical samples. Magnification, x100. Scale bar, 210 um. The quantitative data from all specimens are shown in the bar chart. Each dot in the graph represents an individual clinical sample. Two-sided unpaired Student t test was used to analyze comparisons, and data are presented as means +/- SEM. * $p < 0.05$ and *** $p < 0.001$. (B) Histological analysis of tumor morphology in relation to miR124-3p and miR766-3p target gene expression. Representative images of CREBRF, ATG5, CREB3, NR3C2, β-catenin, and c-Myc expression in the serial section of responder and non-responder HNSCC specimens. BV: Blood Vessel. T: Tumor. N: Normal tissue. The invasive cancer cells are indicated by red arrowhead. Magnification, ×200. Scale bar, 100 um. (C) Summary of resistance mechanisms regulated by miR124-3p and miR766-3p. Our data indicated that upon acquired resistance in HNSCC cells or in non-responder HNSCC tumors, the levels of miR124-3p and miR766-3p go up, which in turn down-regulate its direct target genes: CREBRF and NR3C2, and consequently the expression of downstream targets of CREBRF (ATG5/CREB3) and NR3C2 (β-catenin/c-Myc) increased in resistant tumors, which are positively correlated with poor prognosis. Thus, by enhancing the CREBRF-ATG5/CREB3 and NR3C2-β-catenin/c-Myc axis, miR124-3p and miR766-3p support aggressive HNSCC progression. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/36358691), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Downregulation of CREBRF and NR3C2 increase poor prognosis in HNSCC. (A) Histological analysis of miR124-3p and miR766-3p target gene expression (CREBRF-ATG5/CREB3, NR3C2-β-catenin/c-Myc) in Responder vs. Non-Responder HNSCC clinical samples. Magnification, x100. Scale bar, 210 um. The quantitative data from all specimens are shown in the bar chart. Each dot in the graph represents an individual clinical sample. Two-sided unpaired Student t test was used to analyze comparisons, and data are presented as means +/- SEM. * $p < 0.05$ and *** $p < 0.001$. (B) Histological analysis of tumor morphology in relation to miR124-3p and miR766-3p target gene expression. Representative images of CREBRF, ATG5, CREB3, NR3C2, β-catenin, and c-Myc expression in the serial section of responder and non-responder HNSCC specimens. BV: Blood Vessel. T: Tumor. N: Normal tissue. The invasive cancer cells are indicated by red arrowhead. Magnification, ×200. Scale bar, 100 um. (C) Summary of resistance mechanisms regulated by miR124-3p and miR766-3p. Our data indicated that upon acquired resistance in HNSCC cells or in non-responder HNSCC tumors, the levels of miR124-3p and miR766-3p go up, which in turn down-regulate its direct target genes: CREBRF and NR3C2, and consequently the expression of downstream targets of CREBRF (ATG5/CREB3) and NR3C2 (β-catenin/c-Myc) increased in resistant tumors, which are positively correlated with poor prognosis. Thus, by enhancing the CREBRF-ATG5/CREB3 and NR3C2-β-catenin/c-Myc axis, miR124-3p and miR766-3p support aggressive HNSCC progression. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/36358691), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Park ZM, Sporer A, Kraft K et al. Kar4, the Yeast Homolog of METTL14, is Required for mRNA m (6) A Methylation and Meiosis bioRxiv 2023-08-16 [PMID: 36747717]

Xiong Y, Wang L, Xu S et al. Small molecule Z363 co-regulates TAF10 and MYC via the E3 ligase TRIP12 to suppress tumour growth Clinical and Translational Medicine 2023-01-13 [PMID: 36639831]

T Shibata, DY Cao, TB Dar, F Ahmed, SA Bhat, LC Veiras, EA Bernstein, AA Khan, M Chaum, SL Shiao, WG Tourtellot, JF Giani, KE Bernstein, X Cui, E Vail, Z Khan miR766-3p and miR124-3p Dictate Drug Resistance and Clinical Outcome in HNSCC Cancers, 2022-10-27;14(21):. 2022-10-27 [PMID: 36358691]

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Filidou E, Kandilogiannakis L, Tarapatzi G et al. A Simplified and Effective Approach for the Isolation of Small Pluripotent Stem Cells Derived from Human Peripheral Blood Biomedicines 2023-03-05 [PMID: 36979766] (Immunocytochemistry/ Immunofluorescence, Human)

Liang JH, Akhanov V, Ho A et al. Dopamine signaling from ganglion cells directs layer-specific angiogenesis in the retina Current biology : CB 2023-08-07 [PMID: 37572663]

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Park ZM, Belnap E, Remillard M, Rose MD Vir1p, the Yeast Homolog of Virilizer, is Required for mRNA m 6 A Methylation and Meiosis bioRxiv : the preprint server for biology 2023-02-07 [PMID: 36798303] (Western Blot)

Park Z, Sporer A, Kraft K et al. Kar4, the Yeast Homolog of METTL14, is Required for mRNA m6A Methylation and Meiosis bioRxiv 2023-01-30

Alzaydi MM, Abdul-Salam VB, Whitwell HJ et al. Intracellular Chloride Channels Regulate Endothelial Metabolic Reprogramming in Pulmonary Arterial Hypertension American journal of respiratory cell and molecular biology 2022-10-20 [PMID: 36264759]

Martinez-Velez N, Garcia-Moure M, Marigil M et al. Blood feeding activates the vitellogenic stage of oogenesis in the mosquito Aedes aegypti through inhibition of glycogen synthase kinase 3 by the insulin and TOR pathways Dev Biol 2019-06-03 [PMID: 31153832]

More publications at http://www.novusbio.com/NB600-336

Procedures

Serum protocol for c-Myc Antibody (NB600-336)

Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 40 ug of total protein per lane.
2. Transfer proteins to membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain according to standard Ponceau S procedure (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot.
5. Block the membrane using standard blocking buffer for at least 1 hour.
6. Wash the membrane in wash buffer three times for 10 minutes each.
7. Dilute primary antibody in blocking buffer and incubate 1 hour at room temperature.
8. Wash the membrane in wash buffer three times for 10 minutes each.
9. Apply the diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturers instructions) and incubate 1 hour at room temperature.
10. Wash the blot in wash buffer three times for 10 minutes each (this step can be repeated as required to reduce background).
11. Apply the detection reagent of choice in accordance with the manufacturers instructions.

Note: Tween-20 can be added to the blocking or antibody dilution buffer at a final concentration of 0.05-0.2%.

Immunohistochemistry-Paraffin Embedded Sections

Antigen Unmasking:
Bring slides to a boil in 10 mM sodium citrate buffer (pH 6.0) then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench-top for 30 minutes.

Staining:
1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in wash buffer for 5 minutes.
3. Block each section with 100-400 ul blocking solution for 1 hour at room temperature.
4. Remove blocking solution and add 100-400 ul diluted primary antibody. Incubate overnight at 4 C.
5. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
6. Add 100-400 ul biotinylated diluted secondary antibody. Incubate 30 minutes at room temperature.
7. Remove secondary antibody solution and wash sections three times with wash buffer for 5 minutes each.
8. Add 100-400 ul Streptavidin-HRP reagent to each section and incubate for 30 minutes at room temperature.
9. Wash sections three times in wash buffer for 5 minutes each.
10. Add 100-400 ul DAB substrate to each section and monitor staining closely.
11. As soon as the sections develop, immerse slides in deionized water.
12. Counterstain sections in hematoxylin.
13. Wash sections in deionized water two times for 5 minutes each.
14. Dehydrate sections.
15. Mount coverslips.

Immunocytochemistry Protocol

Culture cells to appropriate density in 35 mm culture dishes or 6-well plates.

1. Remove culture medium and add 10% formalin to the dish. Fix at room temperature for 30 minutes.
2. Remove the formalin and add ice cold methanol. Incubate for 5-10 minutes.
3. Remove methanol and add washing solution (i.e. PBS). Be sure to not let the specimen dry out. Wash three times for 10 minutes.
4. To block nonspecific antibody binding incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
5. Add primary antibody at appropriate dilution and incubate at room temperature from 2 hours to overnight at room

temperature.
6. Remove primary antibody and replace with washing solution. Wash three times for 10 minutes.
7. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
8. Remove antibody and replace with wash solution, then wash for 10 minutes. Add Hoechst 33258 to wash solution at 1:25,0000 and incubate for 10 minutes. Wash a third time for 10 minutes.
9. Cells can be viewed directly after washing. The plates can also be stored in PBS containing Azide covered in Parafilm (TM). Cells can also be cover-slipped using Fluoromount, with appropriate sealing.

*The above information is only intended as a guide. The researcher should determine what protocol best meets their needs. Please follow safe laboratory procedures.

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Products Related to NB600-336

NBL1-13414	c-Myc Overexpression Lysate
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HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control

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