

Product Datasheet

c-Myc Antibody (9E11) - BSA Free NB200-108

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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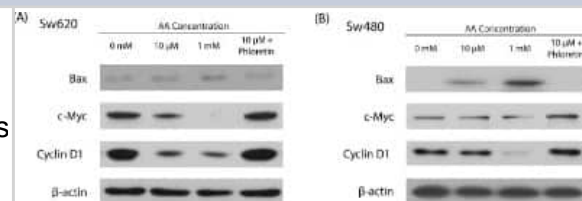
NB200-108

c-Myc Antibody (9E11) - BSA Free

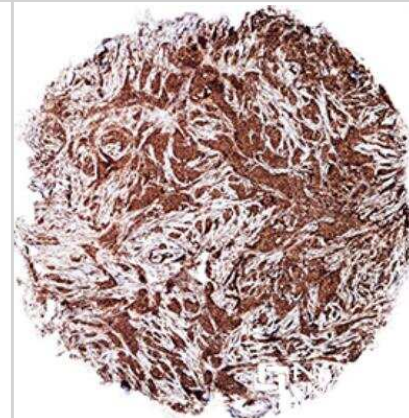
Product Information	
Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	9E11
Preservative	0.02% Sodium Azide
Isotype	IgG2a Kappa
Purity	Protein A or G purified
Buffer	PBS
Target Molecular Weight	48.8 kDa
Product Description	
Host	Mouse
Gene ID	4609
Gene Symbol	MYC
Species	Human, Mouse, Rat, Chicken, Yeast
Reactivity Notes	Rat reactivity reported in scientific literature (PMID: 15777849).
Immunogen	A synthetic peptide corresponding to amino acids 408-420 (AEEQKLISEEDL) of human c-Myc Antibody (9E11), conjugated to KLH. [UniProt# P01106]
Product Application Details	
Applications	Western Blot, ELISA, Flow Cytometry, Flow (Intracellular), Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Immunoprecipitation, Chromatin Immunoprecipitation (ChIP), CyTOF-ready
Recommended Dilutions	Western Blot 1:500-1:1000, Flow Cytometry 1:200-1:400, ELISA 1:100-1:2000, Immunohistochemistry 1:100, Immunocytochemistry/ Immunofluorescence reported in scientific literature (PMID 33542232), Immunoprecipitation 2ug/mg lysate, Immunohistochemistry-Paraffin 1:100, Immunohistochemistry-Frozen 1:100, Flow (Intracellular), Chromatin Immunoprecipitation (ChIP) 2 ug/ 500 ug extract, CyTOF-ready
Application Notes	This antibody is CyTOF ready.

Images

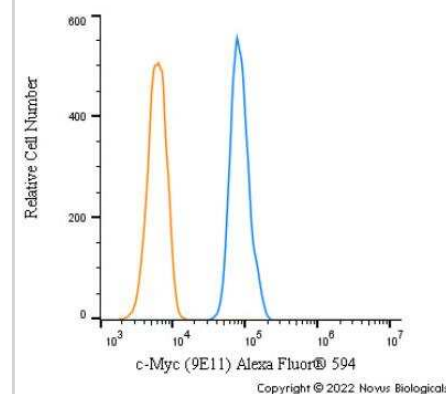
Western Blot: c-Myc Antibody (9E11) [NB200-108] - Expression of cancer proliferation markers and in high SVCT-2 expression cell lines. Western blot analysis of c-Myc and cyclin D1 in Sw620 and Sw480 cell lines after co-treatment with L-ascorbic acid and phloretin. beta-actin was used as a loading control. Image collected and cropped by CiteAb from the following publication (<https://www.nature.com/articles/s41598-018-29386-7>), licensed under a CC-BY license.



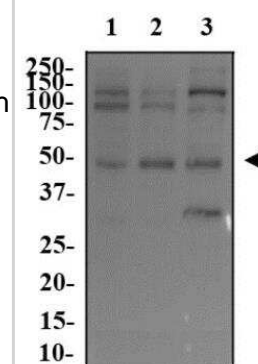
Immunohistochemistry-Paraffin: c-Myc Antibody (9E11) [NB200-108] - c-Myc was detected in immersion fixed paraffin-embedded sections of human breast cancer using anti-human mouse monoclonal antibody (Catalog # NB200-108, clone 9E11) at 1:600 dilution overnight at 4C. Tissue was stained using the VisuCyte anti-mouse HRP polymer detection reagent (Catalog # VC001) with DAB chromogen (brown) and counterstained with hematoxylin (blue). Images may not be copied, printed or otherwise disseminated without express written permission of Novus Biologicals a bio-techne brand.



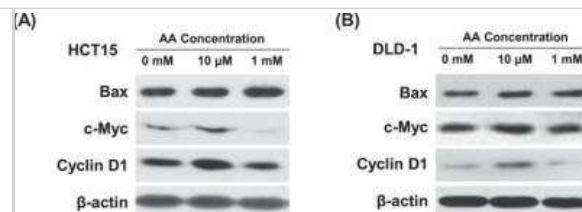
Flow Cytometry: c-Myc Antibody (9E11) [NB200-108] - An intracellular stain was performed on U937 cells with c-Myc [9E11] Antibody NB200-108AF594 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 594.



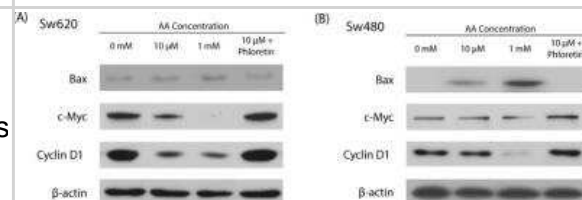
Western Blot: c-Myc Antibody (9E11) [NB200-108] - Whole cell protein from PC3 (lane 1), U-2 OS (lane 2) and mouse testis (lane 3) was separated on a 12% gel by SDS-PAGE, transferred to PVDF membrane and blocked in 5% non-fat milk in TBST. The membrane was probed with 2.0 ug/ml anti-c-Myc in 1% milk, and detected with an anti-mouse HRP secondary antibody using chemiluminescence.



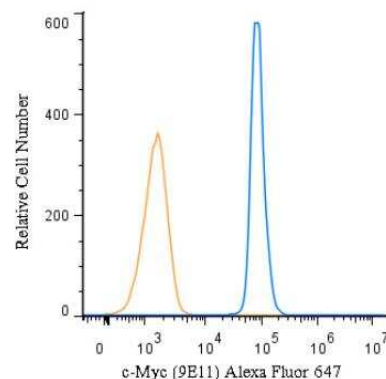
Western Blot: c-Myc Antibody (9E11) [NB200-108] - Expression of cancer proliferation markers in low SVCT-2 expressing cell lines. Expression of c-Myc and cyclin D1 was analyzed by western blotting in HCT15 and DLD-1 cell lines after L-ascorbic acid treatment. beta-actin was used as a loading control. Image collected and cropped by CiteAb from the following publication (<https://www.nature.com/articles/s41598-018-29386-7>), licensed under a CC-BY license.



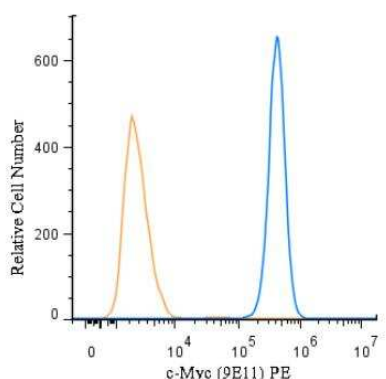
Western Blot: c-Myc Antibody (9E11) [NB200-108] - Expression of cancer proliferation markers and in high SVCT-2 expression cell lines. Western blot analysis of c-Myc and cyclin D1 in Sw620 and Sw480 cell lines after co-treatment with L-ascorbic acid and phloretin. beta-actin was used as a loading control. Image collected and cropped by CiteAb from the following publication (<https://www.nature.com/articles/s41598-018-29386-7>), licensed under a CC-BY license.



Flow (Intracellular): c-Myc Antibody (9E11) [NB200-108] - An intracellular stain was performed on U-937 cells with c-Myc Antibody (9E11) NB200-108AF647 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 647.

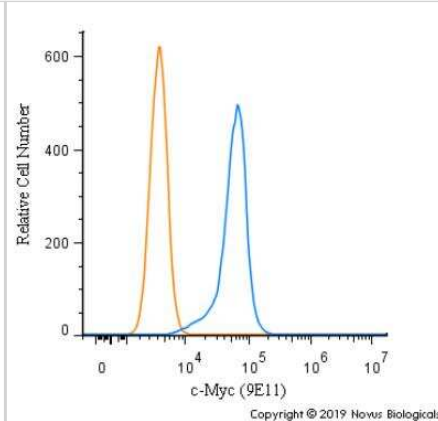


Flow Cytometry: c-Myc Antibody (9E11) [NB200-108] - An intracellular stain was performed on U-937 cells with c-Myc Antibody (9E11) NB200-108PE (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Phycoerythrin.



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Flow Cytometry: c-Myc Antibody (9E11) [NB200-108] - An intracellular stain was performed on U-937 cells with c-Myc Antibody [9E11] NB200-108 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 1.0 ug/mL for 30 minutes at room temperature, followed by Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody.



Publications

Aamir K, Sethi G, Afrin MR et al. Arjunolic acid modulate pancreatic dysfunction by ameliorating pattern recognition receptor and canonical Wnt pathway activation in type 2 diabetic rats Life sciences 2023-08-15 [PMID: 37307966]

Alghamdi TA, Krentz NAJ, Smith N et al. Zmiz1 is required for mature B-cell function and mass expansion upon high fat feeding Molecular metabolism 2022-10-25 [PMID: 36307047] (WB)

Details:

Dilution used in WB 1:200

Cho JH, Okuma A, Sofjan K et al. Engineering advanced logic and distributed computing in human CAR immune cells Nature communications 2021-02-04 [PMID: 33542232] (ICC/IF, Human)

Cho S, Chae JS, Shin H et al. Hormetic dose response to L-ascorbic acid as an anti-cancer drug in colorectal cancer cell lines according to SVCT-2 expression. Sci Rep. 2018-07-27 [PMID: 30054560] (WB, Human)

Hubbi ME, Kshitiz, Gilkes DM et al. A nontranscriptional role for HIF-1alpha as a direct inhibitor of DNA replication. Sci Signal 2013-02-12 [PMID: 23405012]

Matsumura S et al. Activation of the Kaposi's sarcoma-associated herpesvirus major latency locus by the lytic switch protein RTA (ORF50). J Virol 79:8493-505 (2005). [PMID: 15956592] (WB, Human)

Sers JA, Gruppuso PA Nucleolar localization of hepatic c-Myc: a potential mechanism for c-Myc regulation. Biochim Biophys Acta 1743:141-50. 2005-01-01 [PMID: 15777849] (WB, Rat)

Robert F et al. Global position recruitment of HATs HDACs in the yeast genome. Mol Cell 16:199-209. 2004-01-01 [PMID: 15494307]

Zhang H et al. The Yaf9 component of the SWR1 NuA4 complexes is required for proper gene expression, histone H4 acetylation, Htz1 replacement near telomeres. Mol Cell Biol 24:9424-36. 2004-01-01 [PMID: 15485911] (Chemotaxis, Yeast)

Tanaka S, Diffley JF. Interdependent nuclear accumulation of budding yeast Cdt1 and Mcm2-7 during G1 phase. Nat Cell Biol 4 (3):198-207. 2002-03-01 [PMID: 11836525] (IP, Yeast)

Pich A, Margaria E, Chiusa L. Oncogenes and male breast carcinoma: c-erbB-2 and p53 coexpression predicts a poor survival. J Clin Oncol 18(16):2948-56. 2000-08-01 [PMID: 10944127] (IHC-P, Human)

Munchow S, Sauter C, Jansen RP. Association of the class V myosin Myo4p with a localised messenger RNA in budding yeast depends on She proteins. J Cell Sci 112 (Pt 10):1511-8. 1999-05-01 [PMID: 10212145] (IP, Yeast)

Procedures

Western Blot Protocol for c-Myc Antibody (NB200-108)

Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 10-25 ug of total protein per lane.
2. Transfer proteins to PVDF membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain the membrane with Ponceau S (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot TBS -0.05% Tween 20 (TBST).
5. Block the membrane in 5% Non-fat milk in TBST (blocking buffer) for at least 1 hour.
6. Wash the membrane in TBST three times for 10 minutes each.
7. Dilute primary antibody in blocking buffer and incubate overnight at 4C with gentle rocking.
8. Wash the membrane in TBST three times for 10 minutes each.
9. Incubate the membrane in diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturer's instructions) for 1 hour at room temperature.
10. Wash the blot in TBST 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 manufacturer's instructions.

Immunohistochemistry-Paraffin Protocol for c-Myc Antibody (NB200-108)

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 (keep slides in the sodium citrate buffer at all times).

Staining:

1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in PBS for 5 minutes.
3. Block each section with 100-400 ul blocking solution (1% BSA in PBS) 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 HRP polymer conjugated secondary antibody. Incubate 30 minutes at room temperature.
7. Wash sections three times in wash buffer for 5 minutes each.
8. Add 100-400 ul DAB substrate to each section and monitor staining closely.
9. As soon as the sections develop, immerse slides in deionized water.
10. Counterstain sections in hematoxylin.
11. Wash sections in deionized water two times for 5 minutes each.
12. Dehydrate sections.
13. Mount coverslips.



Immunocytochemistry/ Immunofluorescence Protocol for c-Myc Antibody (NB200-108)

Immunocytochemistry Protocol

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

1. Remove culture medium and wash the cells briefly in PBS. Add 10% formalin to the dish and fix at room temperature for 10 minutes.
2. Remove the formalin and wash the cells in PBS.
3. Permeablize the cells with 0.1% Triton X100 or other suitable detergent for 10 min.
4. Remove the permeablization buffer and wash three times for 10 minutes each in PBS. Be sure to not let the specimen dry out.
5. To block nonspecific antibody binding, incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
6. Add primary antibody at appropriate dilution and incubate overnight at 4C.
7. Remove primary antibody and replace with PBS. Wash three times for 10 minutes each.
8. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
9. Remove secondary antibody and replace with PBS. Wash three times for 10 minutes each.
10. Counter stain DNA with DAPI if required.



Flow (Intracellular) Protocol for c-Myc Antibody (NB200-108)

Protocol for Flow Cytometry Intracellular Staining

Sample Preparation.

1. Grow cells to 60-85% confluency. Flow cytometry requires between 2×10^5 and 1×10^6 cells for optimal performance.
2. If cells are adherent, harvest gently by washing once with staining buffer and then scraping. Avoid using trypsin as this can disrupt certain epitopes of interest. If enzymatic harvest is required, use Accutase, Collagenase, or TrypLE Express for a less damaging option.
3. Reserve 100 μ L for counting, then transfer cell volume into a 50 mL conical tube and centrifuge for 8 minutes at 400 RCF.
 - a. Count cells using a hemocytometer and a 1:1 trypan blue exclusion stain to determine cell viability before starting the flow protocol. If cells appear blue, do not proceed.
4. Re-suspend cells to a concentration of 1×10^6 cells/mL in staining buffer (NBP2-26247).
5. Aliquot out 100 μ L samples in accordance with your experimental samples.

Tip: When cell surface and intracellular staining are required in the same sample, it is advisable that the cell surface staining be performed first since the fixation and permeabilization steps might reduce the availability of surface antigens.

Intracellular Staining.

Tip: When performing intracellular staining, it is important to use appropriate fixation and permeabilization reagents based upon the target and its subcellular location. Generally, our Intracellular Flow Assay Kit (NBP2-29450) is a good place to start as it contains an optimized combination of reagents for intracellular staining as well as an inhibitor of intracellular protein transport (necessary if staining secreted proteins). Certain targets may require more gentle or transient permeabilization protocols such as the commonly employed methanol or saponin-based methods.

Protocol for Cytoplasmic Targets:

1. Fix the cells by adding 100 μ L fixation solution (such as 4% PFA) to each sample for 10-15 minutes.
2. Permeabilize cells by adding 100 μ L of a permeabilization buffer to every 1×10^6 cells present in the sample. Mix well and incubate at room temperature for 15 minutes.
 - a. For cytoplasmic targets, use a gentle permeabilization solution such as 1X PBS + 0.5% Saponin or 1X PBS + 0.5% Tween-20.
 - b. To maintain the permeabilized state throughout your experiment, use staining buffer + 0.1% of the permeabilization reagent (i.e. 0.1% Tween-20 or 0.1% Saponin).
3. Following the 15 minute incubation, add 2 mL of the staining buffer + 0.1% permeabilizer to each sample.
4. Centrifuge for 1 minute at 400 RCF.
5. Discard supernatant and re-suspend in 100 μ L of staining buffer + 0.1% permeabilizer.
6. Add appropriate amount of each antibody (eg. 1 test or 1 μ g per sample, as experimentally determined).
7. Mix well and incubate at room temperature for 30 minutes- 1 hour. Gently mix samples every 10-15 minutes.
8. Following the primary/conjugate incubation, add 1-2 mL/sample of staining buffer +0.1% permeabilizer and centrifuge for 1 minute at 400 RCF.
9. Wash twice by re-suspending cells in staining buffer (2 mL for tubes or 200 μ L for wells) and centrifuging at 400 RCF for 5 minutes. Discard supernatant.
10. Add appropriate amount of secondary antibody (as experimentally determined) to each sample.
11. Incubate at room temperature in dark for 20 minutes.
12. Add 1-2 mL of staining buffer and centrifuge at 400 RCF for 1 minute and discard supernatant.
13. Wash twice by re-suspending cells in staining buffer (2 mL for tubes or 200 μ L for wells) and centrifuging at 400 RCF for 5 minutes. Discard supernatant.
14. Resuspend in an appropriate volume of staining buffer (usually 500 μ L per sample) and proceed with analysis on your flow cytometer.



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Products Related to NB200-108

NBL1-13414	c-Myc Overexpression Lysate
HAF007	Goat anti-Mouse IgG Secondary Antibody [HRP]
NB720-B	Rabbit anti-Mouse IgG (H+L) Secondary Antibody [Biotin]
NBP1-96981-0.5mg	Mouse IgG2a Kappa Isotype Control (M2AK)

Limitations

This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

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