Anti-Phospho-c-Myc (T58) Antibody [SN60-01] ET1611-24

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IF-Tissue, FC, IHC-P
Molecular Wt:	Predicted band size: 49 kDa
Clone number:	SN60-01

Description: Myc is a family of regulator genes and proto-oncogenes that code for transcription factors. The Myc family consists of three related human genes: c-myc (MYC), l-myc (MYCL), and n-myc (MYCN). c-myc (also sometimes referred to as MYC) was the first gene to be discovered in this family, due to homology with the viral gene v-myc. In cancer, c-myc is often constitutively (persistently) expressed. This leads to the increased expression of many genes, some of which are involved in cell proliferation, contributing to the formation of cancer. A common human translocation involving c-myc is critical to the development of most cases of Burkitt lymphoma. Constitutive upregulation of Myc genes have also been observed in carcinoma of the cervix, colon, breast, lung and stomach. Myc is thus viewed as a promising target for anti-cancer drugs. Unfortunately, Myc possesses several features that render it undruggable such that any anti-cancer drugs for Myc dysregulation will require acting on the protein indirectly, i.e. targeting the mRNA for the protein rather than a small molecule that targets the protein itself.

Immunogen: Synthetic phospho-peptide corresponding to residues surrounding Thr58 of Human Myc (P01106-1).

Positive control: HCT 116 cell lysate, HCT 116 treated with 25μM MG-132 for 4 hours cell lysate, NIH/3T3 treated with 10μM MG-132 for 8 hours cell lysate, Hela, SKOV-3, human kidney tissue, MCF-7, human skin tissue, mouse skin tissue, rat skin tissue, EL4 cell lysate, EL4 treated with 25μM MG-132 for 4 hours cell lysate.

Subcellular location: Nucleus.

Database links: SwissProt: P01106 Human | P01108 Mouse | P09416 Rat

Recommended Dilutions:

WB	1:1,000
FC	1:50-1:100
IF-Cell	1:50-1:200
IF-Tissue	1:50-1:200
IHC-P	1:500

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

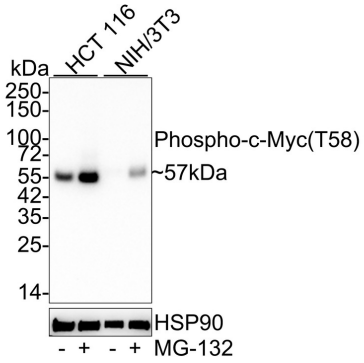

Fig1: Western blot analysis of Phospho-c-Myc (T58) on different lysates with Rabbit anti-Phospho-c-Myc (T58) antibody (ET1611-24) at 1/1,000 dilution.

Lane 1: HCT 116 cell lysate
Lane 2: HCT 116 treated with 25μM MG-132 for 4 hours cell lysate
Lane 3: NIH/3T3 cell lysate
Lane 4: NIH/3T3 treated with 10μM MG-132 for 8 hours cell lysate

Lysates/proteins at 20 μg/Lane.

Predicted band size: 49 kDa
Observed band size: 57 kDa

Exposure time: 2 minutes 18 seconds;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1611-24) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

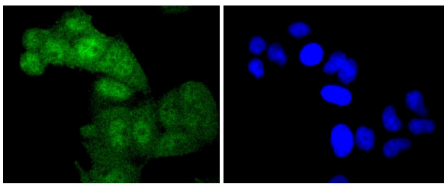

Fig2: ICC staining of Phospho-c-Myc (T58) in Hela cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1611-24, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

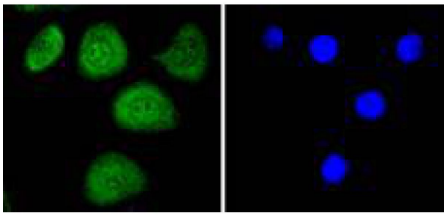

Fig3: ICC staining of Phospho-c-Myc (T58) in SKOV-3 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1611-24, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

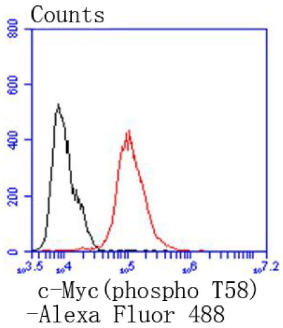

Fig4: Flow cytometric analysis of Phospho-c-Myc (T58) was done on MCF-7 cells. The cells were fixed, permeabilized and stained with the primary antibody (ET1611-24, 1/50) (red). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor 488-conjugated Goat anti-Rabbit IgG Secondary antibody at 1/1,000 dilution for 30 minutes.Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

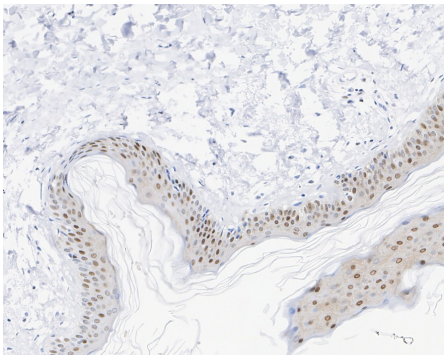

Fig5: Immunohistochemical analysis of paraffin-embedded human skin tissue with Rabbit anti-Phospho-c-Myc (T58) antibody (ET1611-24) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1611-24) at 1/500 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

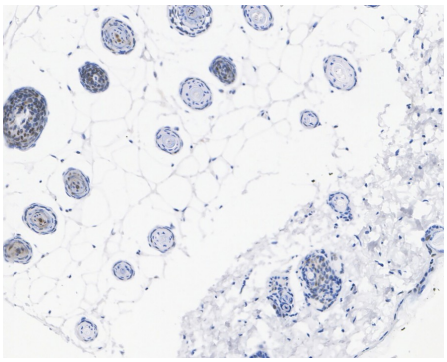

Fig6: Immunohistochemical analysis of paraffin-embedded mouse skin tissue with Rabbit anti-Phospho-c-Myc (T58) antibody (ET1611-24) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1611-24) at 1/500 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

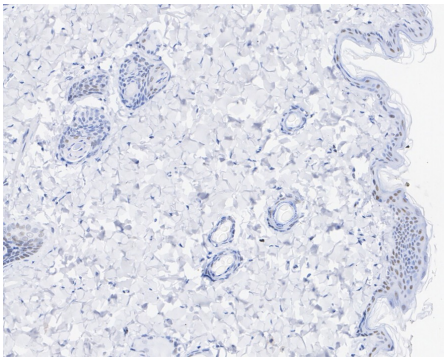

Fig7: Immunohistochemical analysis of paraffin-embedded rat skin tissue with Rabbit anti-Phospho-c-Myc (T58) antibody (ET1611-24) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1611-24) at 1/500 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

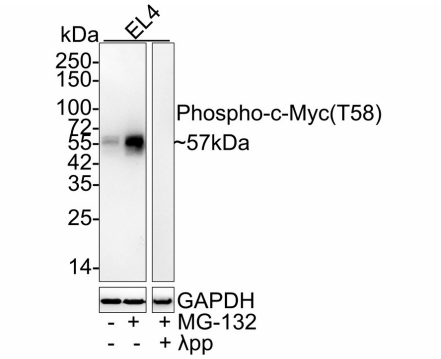

Fig8: Western blot analysis of Phospho-c-Myc (T58) on different lysates with Rabbit anti-Phospho-c-Myc (T58) antibody (ET1611-24) at 1/1,000 dilution.

Lane 1: EL4 cell lysate
Lane 2: EL4 treated with 25μM MG-132 for 4 hours cell lysate
Lane 3: EL4 treated with 25μM MG-132 for 4 hours, then treated with λpp for 1 hour cell lysate

Lysates/proteins at 20 μg/Lane.

Predicted band size: 49 kDa
Observed band size: 57 kDa

Exposure time: 3 minutes;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1611-24) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

Background References

1. Petsalaki, E. et al. 2016. Clks 1, 2 and 4 prevent chromatin breakage by regulating the Aurora B-dependent abscission checkpoint. Nature communications. 7: 11451.
2. Dreer, M. et al. 2016. Interaction of NCOR/SMRT Repressor Complexes with Papillomavirus E8^E2C Proteins Inhibits Viral Replication. PLoS pathogens. 12: e1005556.