Anti-Phospho-c-Myc (S62) Antibody [ST49-08] ET1609-64

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, IP
Molecular Wt:	Predicted band size: 50 kDa
Clone number:	ST49-08

Description: c-Myc-, N-Myc- and L-Myc-encoded proteins function in cell proliferation, differentiation and neoplastic disease. Myc proteins are nuclear proteins with relatively short half lives. Amplification of the c-Myc gene has been found in several types of human tumors including lung, breast and colon carcinomas, while the N-Myc gene has been found amplified in neuroblastomas. The L-Myc gene has been reported to be amplified and expressed at high level in human small cell lung carcinomas. The presence of three sequence motifs in the c-Myc COOH terminus, including the leucine zipper, the helix-loop-helix and a basic region provided initial evidence for a sequence-specific binding function. A basic region helix-loop-helix leucine zipper motif (bHLH-Zip) protein, designated Max, specifically associates with c-Myc, N-Myc and L-Myc proteins. The Myc-Max complex binds to DNA in a sequence-specific manner under conditions where neither Max nor Myc exhibit appreciable binding. Max can also form heterodimers with at least two additional bHLH-Zip proteins, Mad and Mxi1, and Mad-Max dimers have been shown to repress transcription through interaction with mSin3.

Immunogen: Synthetic phospho-peptide corresponding to residues surrounding Ser62 of Human c-Myc aa 46-87 / 439.

Positive control: HepG2 cell lysates, HeLa cell lysates, NIH/3T3 cell lysate, C6 cell lysate, HeLa, rat testis tissue.

Subcellular location: Nucleoplasm, nucleolus.

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

Recommended Dilutions:

WB	1:500-1:2,000
IHC-P	1:50-1:200
IF-Cell	1:50-1:200
IF-Tissue	1:50-1:200
IP	Use at an assay dependent concentration.

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

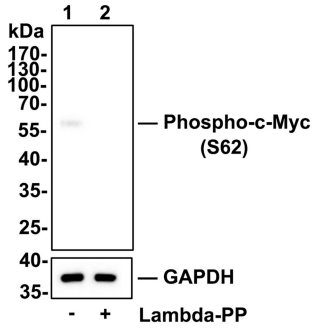

Fig1: Western blot analysis of Phospho-c-Myc(S62) on HepG2 cell lysates.

Lane 1: HepG2 cells, whole cell lysate, 10ug/lane
Lane 2: HepG2 cells treated with 2.8ug/ul lambda-PP for 30 minutes, whole cell lysates, 10ug/lane

All lanes :
Anti-Phospho-c-Myc(S62) antibody (ET1609-64) at 1:500 dilution. Anti-GAPDH antibody (ET1601-4) at 1:10,000 dilution. Goat Anti-Rabbit IgG H&L (HRP) (HA1001) at 1/200,000 dilution.

Predicted band size: 50 kDa
Observed band size: 57 kDa

Blocking and diluting buffer: 5% BSA.

Exposure time: 3 minutes

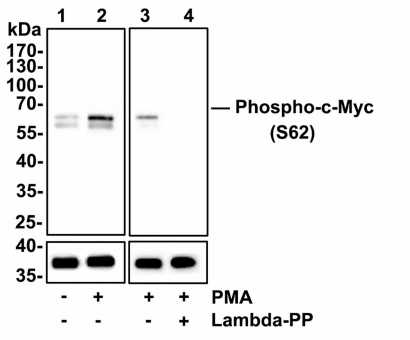

Fig2: Western blot analysis of Phospho-c-Myc(S62) on HeLa cell lysates.

Lane 1: HeLa cells, whole cell lysate, 10ug/lane
Lane 2/3: HeLa cells treated with 200 nM PMA for 10 minutes, whole cell lysates, 10ug/lane
Lane 4: HeLa cells treated with 200 nM PMA for 10 minutes, then treated with 2.8ug/ul lambda-PP for 30 minutes, whole cell lysates, 10ug/lane

All lanes :
Anti-Phospho-c-Myc(S62) antibody (ET1609-64
) at 1:500 dilution. Anti-GAPDH antibody (ET1601-4) at 1:10,000 dilution. Goat Anti-Rabbit IgG H&L (HRP) (HA1001) at 1/200,000 dilution.

Predicted band size: 50 kDa
Observed band size: 57/60 kDa

Blocking and diluting buffer: 5% BSA.

Exposure time: 3 minutes

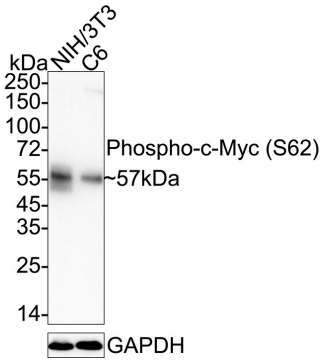

Fig3: Western blot analysis of Phospho-c-Myc (S62) on different lysates with Rabbit anti-Phospho-c-Myc (S62) antibody (ET1609-64) at 1/1,000 dilution.

Lane 1: NIH/3T3 cell lysate
Lane 2: C6 cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 50 kDa
Observed band size: 57 kDa

Exposure time: 3 minutes; ECL: K1801;

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 (ET1609-64) 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.

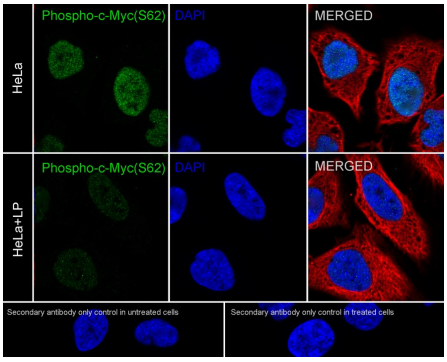

Fig4: Immunocytochemistry analysis of HeLa cells treated with or without λpp labeling Phospho-c-Myc (S62) with Rabbit anti-Phospho-c-Myc (S62) antibody (ET1609-64) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-Phospho-c-Myc (S62) antibody (ET1609-64) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (HA601187, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

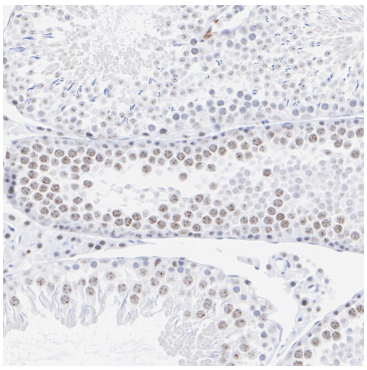

Fig5: Immunohistochemical analysis of paraffin-embedded rat testis tissue with Rabbit anti-Phospho-c-Myc (S62) antibody (ET1609-64) at 1/200 dilution.

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 (ET1609-64) at 1/200 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.

Background References

1. Simons BW et al. A human prostatic bacterial isolate alters the prostatic microenvironment and accelerates prostate cancer progression. J Pathol 235:478-89 (2015).
2. Li S et al. Regulation of c-Myc protein stability by proteasome activator REG . Cell Death Differ 22:1000-11 (2015).