

Anti-cMyc phospho-Ser62 antibody, monoclonal (33A12E10) , Validated

71-161 100ug

Storage temperature: Shipped at 4°C or -20°C and stored at -20°C. Avoid freezing by storing below -25°C

Validation and caution: Specificity of this antibody have been independently validated and caution of the use described in Ref.4, which is recommended to be read by the users..

Reactivity: Human and mouse.

Immunogen: Synthetic peptide containing phospho-Ser62 of cMyc

Applications

1. Western blotting (~1ug/ml, Fig.1)
2. Immunofluorescence staining (0.5~1 µg/ml, Fig.2)
3. Immunohistochemistry (5 µg/ml, Perform heat mediated antigen retrieval with citrate buffer pH 6 before formalin treated paraffin embedded sectioning)
4. Flow cytometry (Use 1 µg for 10⁶ cells)
5. Indirect ELISA (Assay dependent concentration)

Form: Purified monoclonal antibody (IgG) 1mg/ml in PBS, 50% glycerol. Azide- and carrier-free.

Isotype: Mouse IgG2b (κ)

Background: cMyc is a proto-oncogene, which is overexpressed in a wide range of human cancers. Myc gene encodes a transcription factor that regulates a great number of genes through binding on Enhancer Box sequences (E-boxes) and recruiting histone acetyltransferase. It can also act as a transcriptional repressor. It regulates cell growth, apoptosis, differentiation and stem cell self-renewal. Previous studies on the phosphorylation of c-Myc have suggested functional association between phosphorylation at Thr58/Ser62 by glycogen synthase kinase 3, cyclin dependent kinase, ERK2 and C-Jun N terminal Kinase (JNK), cell proliferation and cell cycle regulation. Phosphorylation at Ser62 is required for Ras-induced stabilization and is prerequisite for phosphorylation at Thr58 for its degradation (ref.1).

Data Link UniProtKB/Swiss-Prot [P01106](#) (MYC_HUMAN)

References: This product was used in Ref 1-5 and independently validated in Ref 4.

1. Cianfanelli V *et al.* AMBRA1 links autophagy to cell proliferation and tumorigenesis by promoting c-Myc dephosphorylation and degradation. *Nat Cell Biol* 17:20-30 (2015). [PubMed: 25438055](#) IF, WB (human, mouse)
2. Myant K, *et al.* Serine 62-Phosphorylated MYC Associates with Nuclear Lamins and Its Regulation by CIP2A Is Essential for Regenerative Proliferation. *Cell Rep.* 2015 Aug 11;12(6):1019-31. [PubMed: 26235622](#). WB, IF , IHC (human, mouse)

3. Ischenko I *et al.* Analysis of the tumor-initiating and metastatic capacity of PDX1-positive cells from the adult pancreas. *Proc Natl Acad Sci U S A* 111:3466-71 (2014). [PubMed: 24550494](#) **WB (mouse)**
4. Tibbitts DC *et al.* Studying c-Myc serine 62 phosphorylation in leukemia cells: concern over antibody cross-reactivity. *Blood* 119:5334-5 (2012). [PubMed: 22653959](#) **WB, IP (human)**
5. Tsai WB *et al.* Activation of Ras/PI3K/ERK pathway induces c-Myc stabilization to upregulate argininosuccinate synthetase, leading to arginine deiminase resistance in melanoma cells. *Cancer Res* 72:2622-33 (2012). [PubMed: 22461507](#) **WB (human)**
6. Mathiasen DP. Identification of a c-Jun N-terminal kinase-2-dependent signal amplification cascade that regulates c-Myc levels in ras transformation. *Oncogene*. 2012 Jan 19;31(3):390-401. [PubMed: 21706057](#) **WB (mouse)**
7. Wang X. *et al.* Phosphorylation regulates c-Myc's oncogenic activity in the mammary gland. *Cancer Res*. 2011 Feb 1; 71(3): 925–936. [PubMed: 3077809](#) **WB (human)**
8. Kapeli K & Hurlin PJ Differential Regulation of N-Myc and c-Myc Synthesis, Degradation, and Transcriptional Activity by the Ras/Mitogen-activated Protein Kinase Pathway. *J Biol Chem* 286:38498-508 (2011). [PubMed: 21908617](#) **WB (mouse)**
9. Khanna A. MYC-dependent regulation and prognostic role of CIP2A in gastric cancer. *J Natl Cancer Inst.* 2009 Jun 3;101(11):793-805. [PubMed: 19470954](#) **WB (human)**

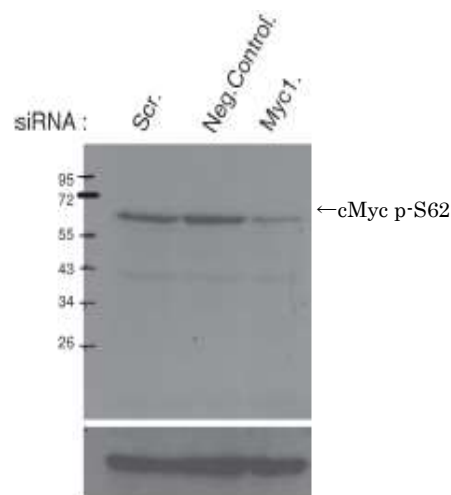


Fig.1. Identification of cMyc protein whose Ser62 is phosphorylated by Western blotting.

Samples: Crude cell extracts of AGS (gastric adenocarcinoma) cells.

Scr; Scrambled siRNA was introduced into the cells as a negative control.

Neg. Control; Negative control siRNA was transfected.

Myc1; siRNA for cMyc was transfected.

(The data was provided by Drs. A. Khanna and J. Westermarck of University of Tampere, Finland)

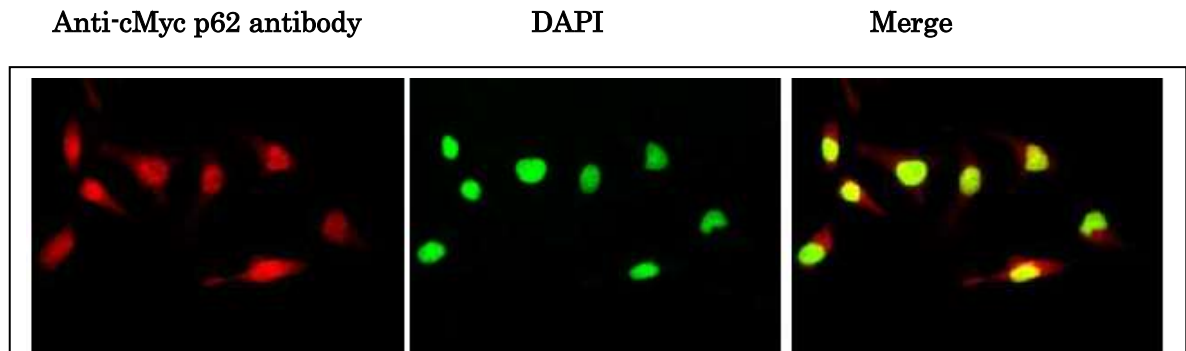


Fig.2. Immunofluorescence staining of cMyc phospho-Ser62 in nuclei of HeLa cells.

1. HeLa cells were fixed with 4% paraformaldehyde overnight, permeabilized with 0.25% Triton X-100 in PBS for 10 min.
2. Incubate cells with 1.5% BSA in PBS for 30 min to block non-specific binding of the antibodies. Incubate the cells with 1/2,000 diluted anti-cMyc p62 antibody in 1% BSA in PBS at 4°C overnight.
3. Incubate cells with a secondary antibody, goat anti-mouse IgG conjugated with Alex 488, at 1/1,000 dilution in 1% BSA for 1 hr at room temperature.
4. Nucleus (DNA) was stained with DAPI

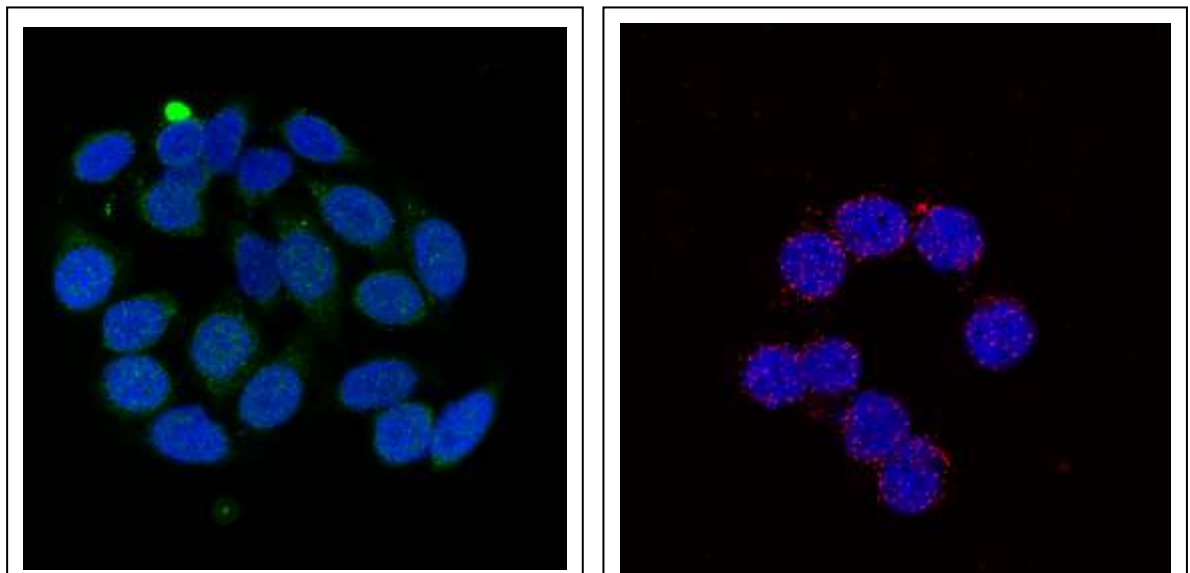


Fig.3. Immunofluorescence staining of cMyc phospho-Ser62 in HeLa cells

Left: Cells stained with anti-cMyc pS62 antibody (green) and DAPI (blue)

Right: Proximity Ligation Analysis with anti-cMyc pS62 and CIP2A antibodies, association of cMyc pS62 with CIP2A (red) in nuclei (DAPI, blue)

Images kindly provided by Prof Westermarck J and Dr. Qiao X. For details refer to Ref.2