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

71-161 100ug

Storage temperature: Shipped at 4℃ or -20℃ and stored at -20℃. Avoid freezing.

Validation: Specificity of this antibody have been independently validated by siRNA

Reactivity: Human and mouse.

Immunogen: Synthetic peptide containing phospho-Ser62 of cMyc

Applications

1. Western blotting (~1ug/ml, Fig.1)
2. Immunofluorescence staining (0.25~1 μg/ml)
3. Immunohistochemistry (5 μg/ml), Paraffin-embedded
4. Flow cytometry (1 μg for 10^6 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)

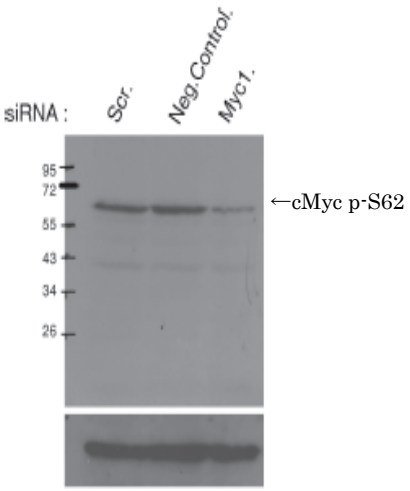

Fig.1. Identification of cMyc protein whose Ser62 is phosphorylated by Western blotting.

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

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

Neg.Contol; 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)

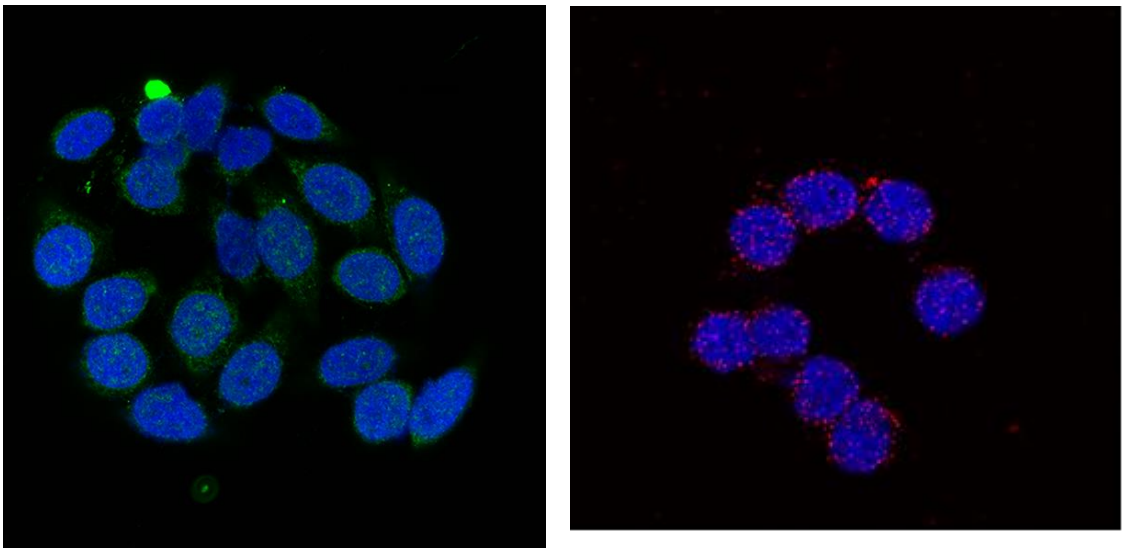

Fig.2. Immunofluorescence staining of cMyc phosho-Ser62 in HeLa cells

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.1

Anti-cMyc p62 antibody DAPI Merge

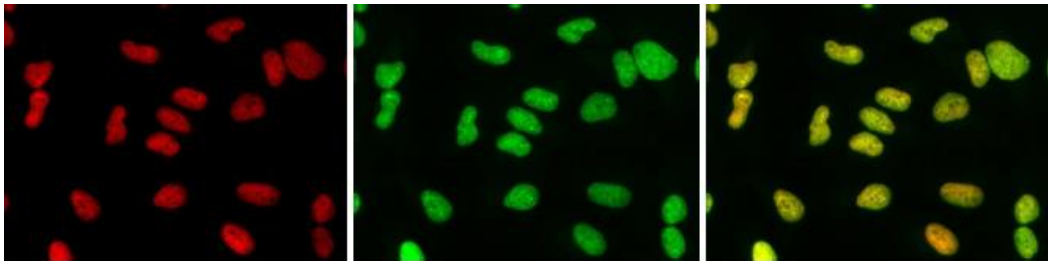

Fig.3. Immunofluorescence staining of cMyc phosho-Ser62 in nuclei of HeLa cells.

1. HeLa cells were fixed with 4% paraformaldehyde overnight, permealized 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 1/4,000 diluted anti-cMyc p62 antibody in 1% BSA in PBS at 4℃ 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

References: This product was used in the following Publications.

1. 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. PMID: 26235622. **WB, IF (human, mouse)**
2. 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)**
3. 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)**
4. 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)**
5. 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)**