

#9401 Store at -20°C

Phospho-c-Myc (Thr58/Ser62) Antibody

✓ 100 µl
(10 Western mini-blot)



Orders ■ 877-616-CELL (2355)
orders@cellsignal.com
Support ■ 877-678-TECH (8324)
info@cellsignal.com
Web ■ www.cellsignal.com

rev. 07/10/08

This product is for *in vitro* research use only and is not intended for use in humans or animals. This product is not intended for use as a therapeutic or in diagnostic procedures.

Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W, F, E-P	H, M, R, Mk	57-70 kDa	Rabbit**

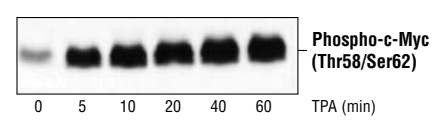
Background: Members of the Myc/Max/Mad network function as transcriptional regulators with roles in various aspects of cell behavior including proliferation, differentiation and apoptosis (1). These proteins share a common basic-helix-loop-helix leucine zipper (bHLH-ZIP) motif required for dimerization and DNA-binding. Max was originally discovered based on its ability to associate with c-Myc and found to be required for Myc's ability to bind DNA and activate transcription (2). Subsequently, Max has been viewed as a central component of the transcriptional network, forming homodimers as well as heterodimers with other members of the Myc and Mad families (1). The association between Max and either Myc or Mad can have opposing effects on transcriptional regulation and cell behavior (1). The Mad family consists of four related protein designated Mad1, Mad2 (Mxi1), Mad3 and Mad4 and more distantly related members of the bHLH-ZIP family, Mnt and Mga. Like Myc, the Mad proteins are tightly regulated with short half-lives. In general, Mad family members interfere with Myc-mediated processes such as proliferation, transformation and prevention of apoptosis by inhibiting transcription (3,4).

Specificity/Sensitivity: Phospho-c-Myc (Thr58/Ser62) Antibody detects endogenous levels of c-Myc singly or doubly phosphorylated at Thr58 and Ser62.

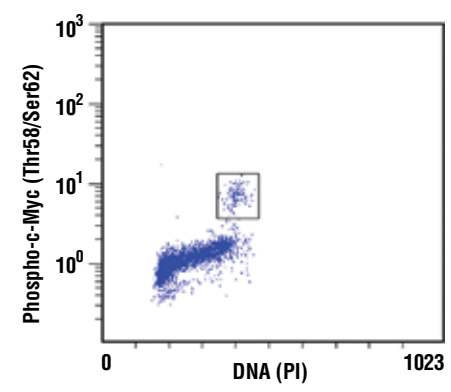
Source/Purification: Polyclonal antibodies are produced by immunizing rabbits with a synthetic phospho-peptide (KLH-coupled) corresponding to residues around Thr58/Ser62 of human c-Myc. Antibodies are purified by protein A and peptide affinity chromatography.

Background References:

- (1) Baudino, T.A. and Cleveland, J.L. (2001) *Mol. Cell Biol.* 21, 691-702.
- (2) Blackwood, E.M. and Eisenman, R.N. (1991) *Science* 251, 1211-1217.
- (3) Henriksson, M. and Luscher, B. (1996) *Adv. Cancer Res.* 68, 109-182.
- (4) Grandori, C. et al. (2000) *Annu. Rev. Cell Dev. Biol.* 16, 653-699.



Western blot analysis of extracts from A431 cells, untreated or TPA-treated, using Phospho-c-Myc (Thr58/Ser62) Antibody.



Flow cytometric analysis of untreated Jurkat cells, using Phospho-c-Myc (Thr58/Ser62) Antibody versus propidium iodide (DNA content). The boxed population indicates phospho-c-Myc-positive cells.

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at -20°C. Do not aliquot the antibody.

*Species cross-reactivity is determined by Western blot.

**Anti-rabbit secondary antibodies must be used to detect this antibody.

Recommended Antibody Dilutions:

Western blotting	1:1000
Flow Cytometry	1:400
ELISA-Peptide	1:1000

Companion Products:

- c-Myc Antibody #9402
- Phospho-p44/42 MAP Kinase (Thr202/Tyr204) Antibody #9101
- Phospho-MEK1/2 (Ser217/221) Antibody #9121
- Phospho-SAPK/JNK (Thr183/Tyr185) Antibody #9251
- Phototope®-HRP Western Blot Detection System, Anti-rabbit IgG, HRP-linked Antibody #7071
- Anti-rabbit IgG, HRP-linked Antibody #7074
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder #7727
- 20X LumiGLO® Reagent and 20X Peroxide #7003

Selected Application References:

- Kamemura, K. et al. (2002) Dynamic interplay between O-glycosylation and O-phosphorylation of nucleocytoplasmatic proteins. *J. Biol. Chem.* 277, 19229-19235. Application: W.
- Noguchi, K. et al. (1999) Regulation of c-Myc through phosphorylation at Ser-62 and Ser-71 by c-Jun N-terminal kinase. *J. Biol. Chem.* 274, 32580-32587. Application: W.
- Sears, R. et al. (2000) Multiple Ras-dependent phosphorylation pathways regulate Myc protein stability. *Genes Dev.* 14, 2501-2514. Application: W.
- Sun, Y. and Clark, E.A. (1999) Expression of the c-myc proto-oncogene is essential for HIV-1 infection in activated T cells. *J. Exp. Med.* 189, 1391-1398. Application: W.
- Watnick, R.S. et al. (2003) Ras modulates Myc activity to repress thrombospondin-1 expression and increase tumor angiogenesis. *Cancer Cell* 3, 219-231. Application: W.

IMPORTANT: For Western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E—ELISA E-P—ELISA Peptide
Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebra fish B—bovine
 Dg—dog Pg—pig Sc—S. cerevisiae All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

Western Immunoblotting Protocol (Primary Antibody Incubation in BSA)

For Western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

A Solutions and Reagents

NOTE: Prepare solutions with Milli-Q or equivalently purified water.

- 1X Phosphate Buffered Saline (PBS)
- 1X SDS Sample Buffer:** 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
- Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
- 10X Tris Buffered Saline (TBS):** To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
- Nonfat Dry Milk (weight to volume [w/v])
- Blocking Buffer:** 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).
- Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
- Bovine Serum Albumin (BSA)
- Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µl Tween-20 (100%).
- Phototope®-HRP Western Blot Detection System #7071:** Includes biotinylated protein ladder, secondary anti-rabbit (#7074) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO® chemiluminescent reagent and peroxide.
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder Detection Pack #7727
- Blotting Membrane:** This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

B Protein Blotting

A general protocol for sample preparation is described below.

- Treat cells by adding fresh media containing regulator for desired time.
- Aspirate media from cultures; wash cells with 1X PBS; aspirate.
- Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
- Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
- Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
- Microcentrifuge for 5 minutes.
- Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

NOTE: CST recommends loading prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.

- Electrotransfer to nitrocellulose or PVDF membrane.

C Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm²) of membrane; for different sized membranes, adjust volumes accordingly.

- (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
- Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.

D Detection of Proteins

- Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO®, 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

NOTE: LumiGLO® substrate can be further diluted if signal response is too fast.

- Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

NOTE: Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO® incubation and declines over the following 2 hours.

Flow Cytometry Protocol for Intracellular Staining Using Conjugated Secondary Antibodies

A Solutions and Reagents

- 1X Phosphate Buffered Saline (PBS):** Dissolve 8 g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4 and 0.24 g KH_2PO_4 in 800 mL distilled water (dH_2O). Adjust the pH to 7.4 with HCl and the volume to 1 liter. Store at room temperature.
- Formaldehyde (methanol free)
- Incubation Buffer:** Dissolve 0.5 g bovine serum albumin (BSA) in 100mL 1X PBS. Store at 4°C

B Fixation

- Collect cells by centrifugation and aspirate supernatant.
- Resuspend cells briefly in 0.5-1 ml PBS. Add formaldehyde to a final concentration of 2-4% formaldehyde.
- Fix for 10 minutes at 37°C.
- Chill tubes on ice for 1 minute.

C Permeabilization

- Permeabilize cells by adding ice-cold 100% methanol slowly to pre-chilled cells, while gently vortexing, to a final concentration of 90% methanol. Alternatively, to remove fix prior to permeabilization, pellet cells by centrifugation and resuspend in 90% methanol.
- Incubate 30 minutes on ice.
- Proceed with staining or store cells at -20°C in 90% methanol.

D Staining Using Unlabeled Primary and Conjugated Secondary Antibodies

NOTE: Allow for isotype matched controls for monoclonal antibodies or species matched IgG for polyclonal antibodies. Count cells using a hemacytometer or alternative method.

- Aliquot $0.5-1 \times 10^6$ cells into each assay tube (by volume).
- Add 2-3 ml Incubation Buffer to each tube and rinse by centrifugation. Repeat.
- Resuspend cells in 100 μl Incubation Buffer per assay tube.
- Block in Incubation Buffer for 10 minutes at room temperature.
- Add the primary antibody at the appropriate dilution to the assay tubes (see individual antibody data sheet for the appropriate dilution).
- Incubate for 30-60 minutes at room temperature.
- Rinse as before in Incubation Buffer by centrifugation.
- Resuspend cells in fluorochrome-conjugated secondary antibody*, diluted in Incubation Buffer according to the manufacturer's recommendations.
- Incubate for 30 minutes at room temperature.
- Rinse as before in Incubation Buffer by centrifugation.
- Resuspend cells in 0.5 ml PBS and analyze on flow cytometer.

*Recommended Secondary Antibodies from Invitrogen.

A-11070 Alexa Fluor® 488 F(ab')₂ fragment of goat anti-rabbit IgG (H+L) (1:1000 dilution)
A-11017 Alexa Fluor® 488 F(ab')₂ fragment of goat anti-mouse IgG (H+L) (1:1000 dilution)

ELISA-Peptide Assay Protocol

A Solutions and Reagents

- Carbonate Buffer:** 15 mM Na_2CO_3 , 35 mM NaHCO_3 , 0.2 g/L NaN_3 (pH 9.6). Use 1 μM synthetic peptide in carbonate buffer.
- 10X Phosphate Buffered Saline (PBS):** To prepare 1 L add 80 g sodium chloride (NaCl), 2 g potassium chloride (KCl), 14.4 g sodium phosphate, dibasic (Na_2HPO_4) and 2.4 g potassium phosphate, monobasic (KH_2PO_4) to 1 L dH_2O . Adjust pH to 7.4.
- Wash Buffer:** 1X PBS containing 0.05% Tween-20 (PBST)
- Blocking Buffer:** 10 mg/ml bovine serum albumin (BSA) in PBST
- Antibody Dilution Buffer:** 3% BSA in PBST
- DELFLIA® Europium-labeled Anti-mouse IgG for mouse primary antibodies or Anti-rabbit IgG (PerkinElmer Life Sciences #AD0124) for rabbit primary antibodies.
- DELFLIA® Enhancement Solution (PerkinElmer Life Sciences #1244-105)

(DELFLIA® is a registered trademark of PerkinElmer, Inc.)

B Protocol

- Coat the wells of a 96-well microtiter plate with 100 μl of 1 μM synthetic peptide in carbonate buffer by incubating overnight at 4°C or for 2 to 6 hours at 37°C. If the peptide does not bind or absorb, try other buffers in the pH 4-8 range.
- Wash plate three times 200 μl /well with wash buffer.
- Block plate with 200 μl /well blocking buffer for 1 hour at 37°C. Wash plate three times with wash buffer. (May leave dry plate at 4°C for 1-2 months if desired.)
- Prepare appropriate dilution of primary antibody with antibody dilution buffer. Add 100 μl to wells and incubate at 37°C for 1 hour.
- Wash three times with wash buffer.
- Add 67 ng/well DELFLIA® Europium-labeled Anti-mouse IgG, diluted in 100 μl /well antibody dilution buffer. Incubate at 37°C for 30 minutes.
- Wash five times with wash buffer.
- Add 100 μl enhancement solution and incubate at 37°C for 15 minutes. Read plate at 615 nm with an appropriate time-resolved plate reader.