

#2861 Store at -20°C

Phospho-c-Abl (Tyr245) Antibody



100 µl
 (10 Western mini-blot)

Orders ■ 877-616-CELL (2355)
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This product is for *in vitro* research use only and is not intended for use in humans or animals.

Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W	H, (M)	135 kDa (c-Abl), 210 kDa (Bcr-Abl)	Rabbit

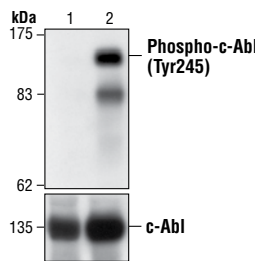
Background: The c-Abl proto-oncogene encodes a nonreceptor type protein tyrosine kinase that is ubiquitously expressed and highly conserved in metazoan evolution. c-Abl protein is distributed in both the nucleus and the cytoplasm of cells. It is implicated in regulating cell proliferation, differentiation, apoptosis, cell adhesion and stress responses (1-3). c-Abl kinase activity is increased *in vivo* by diverse physiological stimuli including integrin activation, PDGF stimulation and binding to c-Jun, Nck and RFX1 (2,4). The *in vivo* mechanism of regulation of c-Abl kinase activity is not completely understood. Tyr245 is located in the linker region between the SH2 and catalytic domains. This positioning is conserved among Abl family members. Phosphorylation of Tyr245 is involved in the activation of c-Abl kinase (5). In addition, phosphorylation of Tyr412 which is located in the kinase activation loop of c-Abl is required for kinase activity (6). Thr735 is located within a conserved 14-3-3 protein binding motif region, and can be phosphorylated upon stress stimulation or TPA treatment (Wu, J. et al. unpublished data). Phosphorylation at Thr735 may play an important role in regulating c-Abl localization as well as its function.

Specificity/Sensitivity: Phospho-c-Abl (Tyr245) Antibody detects endogenous levels of c-Abl only when phosphorylated at Tyr245. The antibody cross-reacts with activated EGF receptors and PDGF receptors.

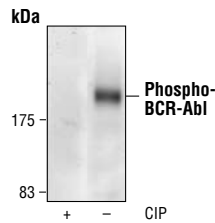
Source/Purification: Polyclonal antibodies are produced by immunizing rabbits with a synthetic phospho-peptide (KLH-coupled) corresponding to residues surrounding Tyr245 of human c-Abl.

Selected Application References:

- Komatsu, N. et al. (2002) A member of Forkhead Transcription Factor FKHL1 Is a Downstream Effector of STI571-induced Cell Cycle Arrest in BCR-ABL-expressing Cells. *J.Biol.Chem.* 278 (8), 6411-6419. Application: W.
- Smith, K.M. et al. (2003) Autoinhibition of Bcr-Abl through its SH3 domain. *Mol. Cell* 12, 27-37. Application: W.



Western blot analysis of immunoprecipitation samples from 293 cells overexpressing Wild-type (lane 1) and constitutively active (lane 2) c-Abl kinase using Phospho-c-Abl (Tyr245) Antibody (upper) or c-Abl Antibody #2862 (lower). (Cell lysates provided by Dr. Giulio Superti-Furga, EMBL, Germany.)



Western blot analysis of K562 leukemia cells, untreated or calf intestinal phosphatase (CIP)-treated using Phospho-c-Abl (Tyr245) Antibody.

Background References:

- (1) Wang, J.Y. et al. (2000) *Oncogene* 19, 5643-5650.
- (2) Van Etten, R.A. et al. (1999) *Trends Cell. Biol.* 9, 179-182.
- (3) Danial, N.N. et al. (2000) *Oncogene* 19, 2523-2531.
- (4) Shaul, Y. et al. (2000) *Cell Death Differ.* 7, 10-16.
- (5) Brasher, B.B. et al. (2000) *J. Biol. Chem.* 275, 35631-35637.
- (6) Pluk, H. et al. (2002) *Cell* 108, 247-259.

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.

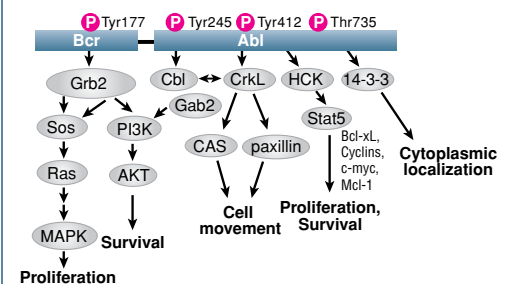
*Species cross-reactivity is determined by Western blot.

Recommended Antibody Dilutions:

Western Blotting 1:1000

Companion Products:

- c-Abl Antibody #2862
- Phospho-c-Abl (Thr735) Antibody #2864
- Phospho-c-Abl (Tyr412) (247C7) Rabbit mAb #2865
- Phospho-c-Abl (Tyr245) (73E5) Rabbit mAb #2868
- 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



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 IC—Immunocytochemistry IF—Immunofluorescence
Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken X—Xenopus
 Species enclosed in parentheses are predicted to react based on 100% sequence homology.

F—Flow cytometry E—ELISA D—DELFIAP®
 Z—zebra fish B—bovine All—all species expected

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.