

Phospho-PP1 α (Thr320) Antibody

100 μ l
(10 Western mini-blot)

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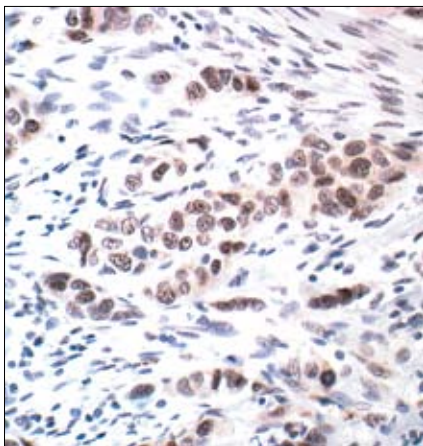
This product is for *in vitro* research use only and is not intended for use in humans or animals.

Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W, IHC-P	H, M, R, Mk	38 kDa	Rabbit

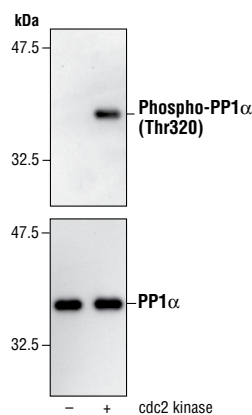
Background: Type 1 protein phosphatase (PP1), a serine/threonine phosphatase, is highly conserved in eukaryotic cells. Four isoforms of PP1 have been characterized: PP1 α , PP1 δ , PP1 γ 1 and PP1 γ 2 (1). Involvement in cell cycle regulation is one of the biological functions of PP1 (1). It has been illustrated that PP1 dephosphorylates Rb and cdc25 during mitosis (2,3). A cell cycle-dependent phosphorylation at Thr320 of PP1 α by cdc2 kinase inhibits PP1 α activity (4).

Specificity/Sensitivity: Phospho-PP1 α (Thr320) Antibody detects PP1 α only when phosphorylated at Thr 320. This antibody may cross-react with the phosphorylated δ or γ isoforms of PP1.

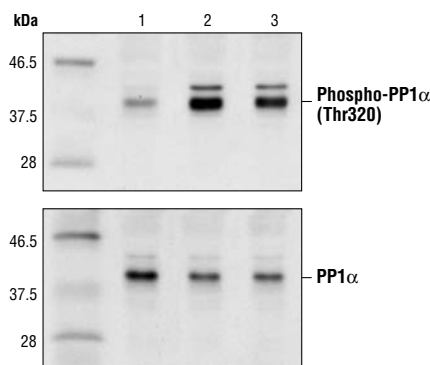
Source/Purification: Polyclonal antibodies are produced by immunizing rabbits with a synthetic phospho-peptide (KLH-coupled) corresponding to residues surrounding Thr320 of human PP1 α . Antibodies are purified by protein A and peptide affinity chromatography.



Immunohistochemical analysis of paraffin-embedded human breast carcinoma, showing nuclear localization, using Phospho-PP1 α (Thr320) Antibody.



Western blot analysis of Recombinant PP1 α , incubated with or without cdc2 protein kinase in the presence of ATP and kinase assay buffer at 30°C for 30 minutes, using Phospho-PP1 α (Thr320) Antibody (upper) or control PP1 α Antibody #2582 (lower).



Western blot analysis of extracts from HeLa cells, asynchronous (lane 1), G2/M (lane 2) or released from G2/M for 6 hours (lane 3), using Phospho-PP1 α (Thr320) Antibody (upper) or PP1 α Antibody #2582 (lower).

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 μ g/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
Immunohistochemistry (Paraffin) 1:50

Companion Products:

PP1 α Antibody #2582
Rb-C Fusion Protein #6022
PhosphoPlus® cdc2 (Tyr15) Antibody Kit #9110
Phospho-cdc2 (Tyr15) Antibody #9111
cdc2 Antibody #9112
cdc2 (Tyr15) Control Proteins #9113
PhosphoPlus® Rb (Ser780, Ser795, Ser807/811) Antibody Kit #9300
Phospho-Rb (Ser795) Antibody #9301
Rb Control Proteins #9303
Phospho-Rb (Ser780) Antibody #9307
Phospho-Rb (Ser807/811) Antibody #9308
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 Detection Pack #7727
20X LumiGLO® Reagent and 20X Peroxide #7003

Selected Application References:

Broceno, C. et al. (2002) RB activation defect in tumor cell lines RB activation defect in tumor cell lines. *PNAS* 99 (22), 14200–14205. Application: W.

Background References:

- (1) Rubin, E. et al. (1998) *Front. Biosci.* 3, D1209-1219.
- (2) Durfee, T. et al. (1993) *Genes Dev.* 7, 555-569.
- (3) Izumi, T. et al. (1992) *Mol. Cell. Biol.* 3, 927-939.
- (4) Kwon, Y. G. et al. (1997) *Proc. Natl. Acad. Sci. USA* 94, 2168-2173.

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—DELFIATM
Z—zebra fish B—bovine All—all species expected

Western Immunoblotting Protocol (Primary Ab 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.

Immunohistochemistry Protocol for Paraffin Sections

A Solutions and Reagents

- 10X Phosphate Buffered Saline (PBS):** 0.58 M sodium phosphate dibasic (Na_2HPO_4), 0.17 M sodium phosphate monobasic (NaH_2PO_4), 0.68 M NaCl. To prepare 1 liter of 10X PBS, combine 82.33 g Na_2HPO_4 , 23.45 g $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ and 40 g NaCl. Adjust pH to 7.4.
- 10 mM Sodium Citrate Buffer:** To prepare 1 liter, add 2.94 g sodium citrate to 1 liter distilled H_2O (dH_2O). Adjust pH to 6.0.
- EDTA (optional)*
- 1% Hydrogen Peroxide:** To prepare, add 10 ml 30% H_2O_2 to 290 ml dH_2O .
- Blocking Solution:** 5% horse serum or goat serum in PBS
- ABC Reagent:** (Vectastain ABC Kit, Vector Laboratories, Inc., Burlingame, CA) Prepare according to manufacturer's instructions 30 minutes before use.
- DAB Reagent:** Add 6.7 μl of 30% hydrogen peroxide to 10 ml dH_2O ; add this mixture to 10 ml of 1 mg/ml DAB (diaminobenzidine tetrahydrochloride) in PBS, filter.

B Protocol

1. Deparaffinize/hydrate sections:

- Incubate sections in three washes of xylene for 5 minutes each.
- Incubate sections in two washes of 100% ethanol for 10 minutes each.
- Incubate sections in two washes of 95% ethanol for 10 minutes each.

- Wash sections twice in dH_2O for 5 minutes each.
- Wash sections in PBS for 5 minutes.
- For antigen unmasking, heat sections in 10 mM sodium citrate buffer (pH 6.0) for 1 minute at full power followed by 9 minutes at medium power.* (Keep slides fully immersed in buffer and maintain temperature at or just below boiling.) Cool slides for 20 minutes after antigen unmasking.

* Alternatively in Step B4, use 1 mM EDTA (pH 8.0), which gives superior results for EGF receptor and HER2/ErbB2 antibodies. See the Tyrosine Kinases/Docking Proteins section of the catalog for these products.

- Wash sections in dH_2O three times for 5 minutes each.
- Incubate sections in 1% Hydrogen Peroxide for 10 minutes.
- Wash sections in dH_2O three times for 5 minutes each.
- Wash section in PBS for 5 minutes.
- Block each section with 100–400 μl blocking solution for 1 hour at room temperature.
- Remove blocking solution and add 100–400 μl diluted primary antibody to each section. (Dilute antibody in blocking solution.) Incubate overnight at 4°C.
- Remove antibody solution and wash sections in PBS three times for 5 minutes each.
- Add 100–400 μl secondary antibody, diluted in blocking solution, to each section. Incubate 30 minutes at room temperature.
- If using ABC avidin/biotin method, make ABC reagent according to the manufacturer's instructions and incubate solution for 30 minutes at room temperature.
- Remove secondary antibody solution and wash sections three times with PBS for 5 minutes each.
- Add 100–400 μl ABC reagent to each section and incubate for 30 minutes at room temperature.
- Remove ABC reagent and wash sections three times in PBS for 5 minutes each.
- Add 100–400 μl DAB reagent to each section and monitor staining closely.
- As soon as the section turns brown, immerse slides in dH_2O .
- If desired, counterstain sections in hematoxylin for 10 seconds.
- Wash sections in dH_2O two times for 5 minutes each.
- Dehydrate sections:**
 - Incubate sections in 95% ethanol two times for 10 seconds each.
 - Repeat in 100% ethanol, incubating sections two times for 10 seconds each.
 - Repeat in xylene, incubating sections two times for 10 seconds each.
- Mount coverslips.