

Phospho-PTP α (Tyr789) Antibody

✓ 100 μ l
(10 Western mini-blot)



Cell Signaling
TECHNOLOGY®

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

Applications W, IP	Species Cross-Reactivity H, M, R, Mk	Molecular Wt. 145 kDa	Source Rabbit
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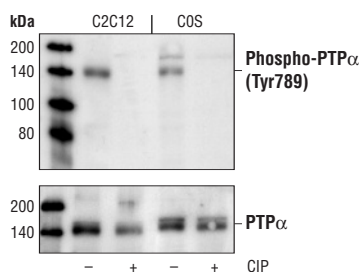
Background: PTP α is a transmembrane receptor tyrosine phosphatase expressed in a variety of tissues. Its cytoplasmic region contains two catalytic domains, but only one exhibits significant activity (1). PTP α is a physiological regulator of Src and Src family members (2). Phosphorylation of Tyr789 at the carboxy-terminus of PTP α is crucial for its specific dephosphorylation of Src at Tyr527, and it is capable of binding to the SH2 domain of either GRB2 or Src (3,4). PTP α activity is stimulated by PKC phosphorylation at Ser180/204 (4,5). Dimerization and association with other PTPs inhibits PTP α activity (6,7).

Specificity/Sensitivity: Phospho-PTP α (Tyr789) Antibody detects endogenous levels of PTP α only when phosphorylated at tyrosine 789. This antibody does not cross-react with other phosphorylated receptor tyrosine phosphatases.

Source/Purification: Polyclonal antibodies are produced by immunizing rabbits with a synthetic phosphopeptide (KLH-coupled) corresponding to residues surrounding Tyr789 of human PTP α . Antibodies are purified by protein A and peptide affinity chromatography.

Background References:

- (1) den Hertog, J. (1999) *Mech. Dev.* 85, 3–14.
- (2) den Hertog, J. et al. (1993) *EMBO J.* 12, 3789–3798.
- (3) Zheng, X. et al. (2000) *EMBO J.* 19, 964–978.
- (4) Zheng, X. et al. (2002) *J. Biol. Chem.* 277, 21922–21929.
- (5) Tracy, S. et al. (1995) *J. Biol. Chem.* 270, 10587–10594.
- (6) Jiang, G. et al. (1999) *Nature* 401, 606–610.
- (7) Blanchetot, C. and den Hertog, J. (2000) *J. Biol. Chem.* 275, 12446–12452.



Western blot analysis of untreated or alkaline phosphatase (CIP)-treated C2C12 and Cos cell lysates, using Phospho-PTP α (Tyr789) Antibody (upper) or PTP α Antibody (Gift from Dr. D. Shalloway, Dept. of Molecular Biology and Genetics, Cornell University, Ithaca, NY.) (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.

Recommended Antibody Dilutions:

Western blotting 1:1000
Immunoprecipitation 1:50

Companion Products:

- Phospho-Src Family (Tyr416) Antibody #2101
- Nonphospho-Src (Tyr416) (7G9) Monoclonal Antibody #2102
- Phospho-Src (Tyr527) Antibody #2105
- Nonphospho-Src (Tyr527) Antibody #2107
- Phospho-PKC (pan) Antibody #9371
- Phospho-PKC α / β II (Thr638/641) Antibody #9375
- Phospho-PKC δ / θ (Ser643/676) Antibody #9376
- Phospho-PKC δ (Thr505) Antibody #9374
- Phospho-PKC θ (Thr538) Antibody #9377
- Phospho-PKC ζ / λ (Thr410/403) Antibody #9378
- GRB2 Antibody #3972
- Phototope®-HRP Western 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
- LumiGLO® Reagent and Peroxide #7003

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

Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry IC—Immunocytochemistry F—Flow cytometry E—ELISA D—DELFIATM
Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken X—Xenopus Z—zebra fish All—all species expected
Species enclosed in parentheses are predicted to react based on 100% sequence homology.

Western Immunoblotting Protocol

For Western blots, incubate membrane with diluted anti-body in 5% 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.

- A1. ■ 1X Phosphate Buffered Saline (PBS)
- A2. ■ 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
- A3. ■ Transfer Buffer:
25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
- A4. ■ 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).
- A5. ■ Nonfat Dry Milk (weight to volume [w/v])
- A6. ■ 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%).
- A7. ■ Wash Buffer:
1X TBS, 0.1% Tween-20 (TBS/T)
- A8. ■ Bovine Serum Albumin (BSA)
- A9. ■ 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%).
- A10. ■ 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.
- A11. ■ Prestained Protein Marker, Broad Range (Premixed Format) #7720
- A12. ■ Biotinylated Protein Ladder Detection Pack #7727
- A13. ■ 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.

- B1. Treat cells by adding fresh media containing regulator for desired time.
- B2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
- B3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
- B4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
- B5. Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
- B6. Microcentrifuge for 5 minutes.
- B7. 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.

- B8. 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.

- C1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
- C2. Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
- C3. Wash three times for 5 minutes each with 15 ml of TBS/T.
- C4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- C5. Wash three times for 5 minutes each with 15 ml of TBS/T.
- C6. 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.
- C7. Wash three times for 5 minutes each with 15 ml of TBS/T.

D. Detection of Proteins

- D1. 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.

- D2. 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.

A. Solutions and Reagents

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

A1. ■ 1X Phosphate Buffered Saline (PBS)

A2. ■ 1X Cell Lysis Buffer:
20 mM Tris (pH 7.5)
150 mM NaCl
1 mM EDTA
1 mM EGTA
1% Triton X-100
2.5 mM Sodium pyrophosphate
1 mM β -Glycerolphosphate
1 mM Na_3VO_4
1 $\mu\text{g}/\text{ml}$ Leupeptin

Note: CST recommends adding 1 mM PMSF before use.*

A3. ■ Transfer Buffer:
25 mM Tris base, 0.2 mM glycine, 20% methanol (pH 8.5)

A4. ■ Protein A Agarose Beads:
(Can be stored for 2 weeks at 4°C.) Add 5 ml of 1X PBS to 1.5 g of protein A agarose beads. Shake 2 hours at 4°C; spin down. Wash pellet twice with PBS. Resuspend beads in 1 volume of PBS.

A5. ■ 3X SDS Sample Buffer:
187.5 mM Tris-HCl (pH 6.8 at 25°C), 6% w/v SDS, 30% glycerol, 150 mM DTT, 0.03% w/v bromophenol blue

B. Preparing Cell Lysates

- B1. Aspirate media. Treat cells by adding fresh media containing regulator for desired time.
- B2. To harvest cells under nondenaturing conditions, remove media and rinse cells once with ice-cold PBS.
- B3. Remove PBS and add 0.5 ml 1X ice-cold cell lysis buffer plus 1 mM PMSF* to each plate (10 cm) and incubate the plate on ice for 5 minutes.
- B4. Scrape cells off the plate and transfer to microcentrifuge tubes. Keep on ice.
- B5. Sonicate on ice four times for 5 seconds each.
- B6. Microcentrifuge for 10 minutes at 4°C, and transfer the supernatant to a new tube. The supernatant is the cell lysate. If necessary, lysate can be stored at -80°C.

C. Immunoprecipitation

- C1. Take 200 μl cell lysate and add primary antibody; incubate with gentle rocking overnight at 4°C.
- C2. Add protein A agarose beads (20 μl of 50% bead slurry). Incubate with gentle rocking for 1–3 hours at 4°C.
- C3. Microcentrifuge for 30 seconds at 4°C. Wash pellet five times with 500 μl of 1X cell lysis buffer. Keep on ice during washes.
- C4. Resuspend the pellet with 20 μl 3X SDS sample buffer. Vortex, then microcentrifuge for 30 seconds.
- C5. Heat the sample to 95–100°C for 2–5 minutes.
- C6. Load the sample (15–30 μl) on SDS-PAGE gel (12–15%).
- C7. Analyze sample by Western blotting (see Western Immunoblotting Protocol).