Store at -20°C

#9921

Phospho-PKC Antibody Sampler Kit

✓ 1 Kit
(9 x 40 µl)

Orders ■ 877-616-CELL (2355)
orders@cellsignal.com
Support ■ 877-678-TECH (8324)
info@cellsignal.com
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This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Phospho-PKD/PKCµ (Ser916) Antibody	2051	40 µl	115 kDa	Rabbit IgG
PKD/PKCµ Antibody	2052	40 µl	115 kDa	Rabbit IgG
Phospho-PKD/PKCµ (Ser744/748) Antibody	2054	40 µl	115 kDa	Rabbit IgG
Phospho-PKC (pan) (βII Ser660) Antibody	9371	40 µl	78, 80, 82, 85 kDa	Rabbit IgG
Phospho-PKCα/β II (Thr638/641) Antibody	9375	40 µl	80, 82 kDa	Rabbit IgG
Phospho-PKCδ (Thr505) Antibody	9374	40 µl	78 kDa	Rabbit IgG
Phospho-PKCδ/θ (Ser643/676) Antibody	9376	40 µl	78 kDa	Rabbit IgG
Phospho-PKCθ (Thr538) Antibody	9377	40 µl	79 kDa	Rabbit IgG
Phospho-PKCζ/λ (Thr410/403) Antibody	9378	40 µl	76 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.

Description: Phospho-PKC Antibody Sampler Kit contains 40 µl of each primary antibody and 100 µl of anti-rabbit IgG secondary antibody (HRP-conjugate).

Background: Activation of protein kinase C (PKC) is one of the earliest events in a cascade that controls a variety of cellular responses, including secretion, gene expression, proliferation and muscle contraction (1,2). PKC isoforms belong to three groups based on calcium dependency and activators. Classical PKCs are calcium-dependent via their C2 domains and are activated by phosphatidylserine (PS), diacylglycerol (DAG) and phorbol esters (TPA, PMA) through their cysteine-rich C1 domains. Both novel and atypical PKCs are calcium-independent, but only novel PKCs are activated by PS, DAG and phorbol esters (3-5). Members of these three PKC groups contain a pseudo-substrate or autoinhibitory domain that binds to substrate-binding site in the catalytic domain to prevent activation in the absence of cofactors or activators.

Control of PKC activity is regulated through three distinct phosphorylation events. Phosphorylation of Thr500 in the activation loop, the autophosphorylation site at Thr641 and at carboxy-terminal hydrophobic site Ser660 occurs *in vivo* (2). Atypical PKC isoforms lack hydrophobic region phosphorylation, which correlates with the presence of glutamic acid rather than the serine or threonine residues found in more typical PKC isoforms. Either the enzyme PDK1 or a close relative is responsible for PKC activation.

A recent addition to the PKC superfamily is PKCµ (PKD), which is regulated by DAG and TPA through its C1 domain. PKD is distinguished by the presence of a PH domain and by its unique substrate recognition and Golgi localization (6). PKC-related kinases (PRK) lack the C1 domain and do not respond to DAG or phorbol esters. Phosphatidylinositol lipids activate PRKs and small Rho-family GTPases bind to the homology region 1 (HR1) to regulate PRK kinase activity (7).

Specificity/Sensitivity: Phospho-PKC (pan) (βII Ser660) Antibody detects PKCα, β I, β II, δ, ε and η isoforms only when phosphorylated at a carboxy-terminal residue homologous to Ser660 of PKCβ II. Phospho-PKCδ (Ser643) Antibody detects PKCδ when phosphorylated at Ser643, and PKCθ when phosphorylated at Ser676. PKD/PKCµ antibody detects PKD/PKCµ only. All other phospho-PKC antibodies recognize only their specific isoform when phosphorylated at the indicated sites.

Source/Purification: Polyclonal antibodies are produced by immunizing animals with synthetic phospho-peptides (KLH-coupled) derived from the sequence of the human protein PKCβ II, PKCα, PKCδ, PKCθ, PKCζ or the mouse protein (PKD). Antibodies are purified by protein A and peptide affinity chromatography.

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 antibodies.*

Recommended Antibody Dilutions:
Western blotting 1:1000

Please visit www.cellsignal.com for a complete listing of recommended companion products.

Background References:

(1) Nishizuka, Y. (1984) *Nature* 308, 693–698.

(2) Keranen, L.M. et al. (1995) *Curr. Biol.* 5, 1394–1403.

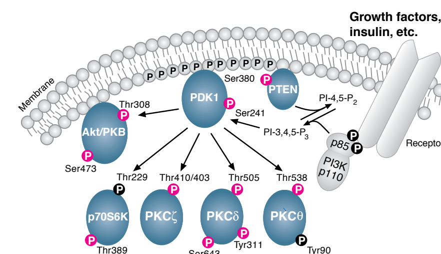
(3) Mellor, H. and Parker, P.J. (1998) *Biochem J.* 332 (Pt 2), 281–292.

(4) Ron, D. and Kazanietz, M.G. (1999) *FASEB J.* 13, 1658–1676.

(5) Moscat, J. and Diaz-Meco, M.T. (2000) *EMBO Rep.* 1, 399–403.

(6) Baron, C.L. and Malhotra, V. (2002) *Science* 295, 325–328.

(7) Flynn, P. et al. (2000) *J. Biol. Chem.* 275, 11064–11070.



Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **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.

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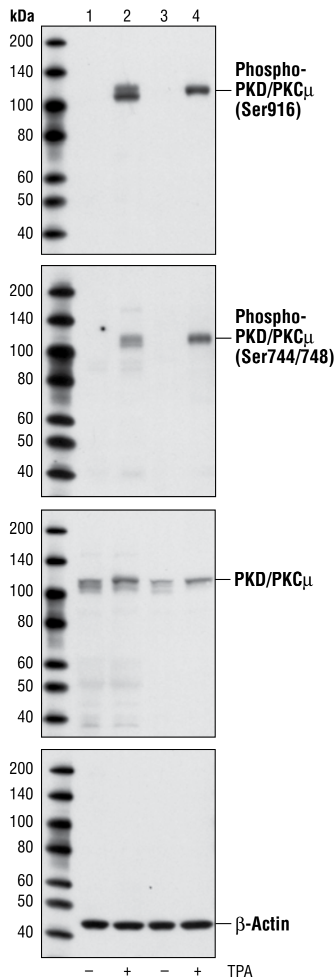
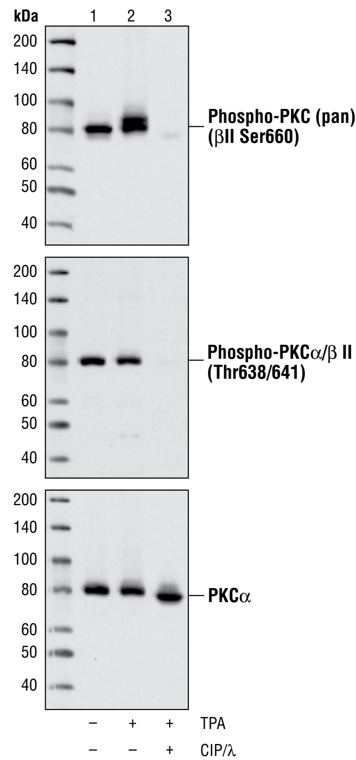
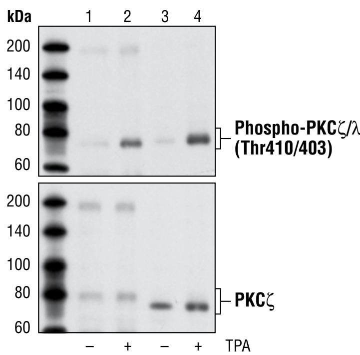


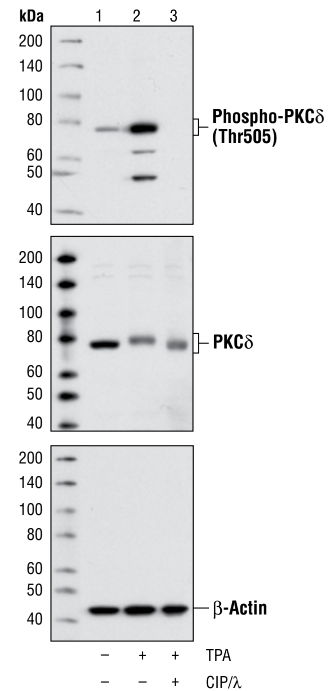
Western blot analysis of extracts from C6 cells (lanes 1 & 2) and COS cells (lanes 3 & 4), untreated (lanes 1 & 3), or TPA-treated (.2μM for 20 minutes) (lanes 2 & 4), using ***Phospho-PKD/PKCμ (Ser916) Antibody #2051****,* ***Phospho-PKD/PKCμ (Ser744/748) Antibody #2054****,* ***PKD/PKCμ Antibody #2052****, and* ***β-actin Antibody #4967.***



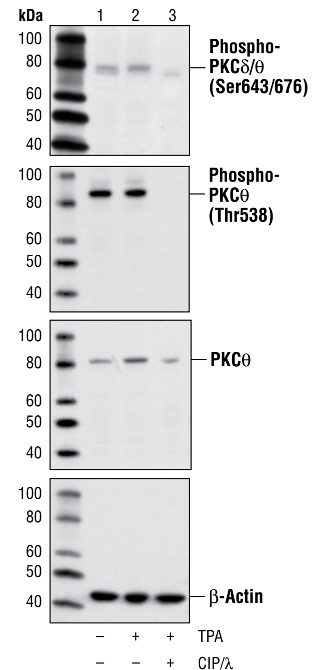
Western blot analysis of extracts from 293 cells, untreated (lane 1), TPA-treated (.2μM for 20 minutes) (lanes 2 & 3), and CIP/λ phosphatase treated (lane 3), using ***Phospho-PKC (pan) (βII Ser660) Antibody #9371****,* ***Phospho-PKCα/βII (Thr638/641) Antibody #9375****, and* ***PKCα Antibody #2056****.*



Western blot analysis of extracts from NIH3T3 cells (lanes 1 & 2) and HeLa cells (lanes 3 & 4), untreated (lanes 1 & 3), PGDF-treated (lane 2), and TNFα-treated (lane 4), using ***Phospho- PKCζ/λ (Thr410/403) Antibody #9378****, and* ***PKCζ Antibody #9368****.*



Western blot analysis of extracts from HeLa cells, untreated (lane 1), TPA-treated (0.2 μM for 20 minutes) (lanes 2 & 3), and CIP/λ phosphatase treated (lane 3), using ***Phospho-PKCδ (Thr505) Antibody #9374****,* ***PKCδ Antibody #2058****, and* ***β-actin Antibody #4967****.*



Western blot analysis of extracts from 293 cells, untreated (lane 1), TPA-treated (.2μM for 20 minutes) (lanes 2 & 3), and CIP/λ phosphatase treated (lane 3), using ***Phospho-PKCδ/θ (Ser643/676) Antibody #9376****,* ***Phospho-PKCθ (Thr538) Antibody #9377****,* ***PKCθ Antibody #2059,*** *and* ***β-actin Antibody #4967****.*

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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.

1. 1X Phosphate Buffered Saline (PBS)
2. **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
3. **Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
4. **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).
5. Nonfat Dry Milk (weight to volume [w/v])
6. **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%).
7. **Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
8. Bovine Serum Albumin (BSA)
9. **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%).
10. **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.
11. Prestained Protein Marker, Broad Range (Premixed Format) #7720
12. Biotinylated Protein Ladder Detection Pack #7727
13. **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.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. 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.
4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
5. Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
6. Microcentrifuge for 5 minutes.
7. 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.

8. Electrotransfer to nitrocellulose or PVDF membrane.

C Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm^2) of membrane; for different sized membranes, adjust volumes accordingly.

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
3. Wash three times for 5 minutes each with 15 ml of TBS/T.
4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
5. Wash three times for 5 minutes each with 15 ml of TBS/T.
6. 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.
7. Wash three times for 5 minutes each with 15 ml of TBS/T.

D Detection of Proteins

1. 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.

2. 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.

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