

# PKC Isoform Antibody Sampler Kit



1 Kit  
 (4 x 40 µl)

**Orders** ■ 877-616-CELL (2355)  
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**Support** ■ 877-678-TECH (8324)  
 info@cellsignal.com  
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New 08/07

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

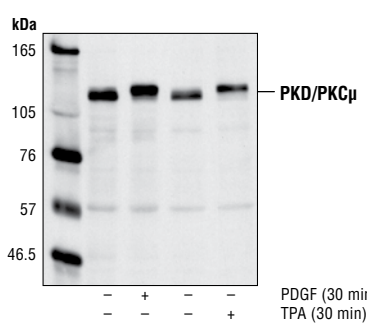
| Products Included                    | Product # | Quantity | Applications    | Species Cross-Reactivity | Mol. Wt. | Source |
|--------------------------------------|-----------|----------|-----------------|--------------------------|----------|--------|
| PKCα Antibody                        | 2056      | 40 µl    | W, IP, IF-IC, F | H, M, R, Mk              | 80 kDa   | Rabbit |
| PKCδ Antibody                        | 2058      | 40 µl    | W, IP           | H, M, R, Mk              | 78 kDa   | Rabbit |
| PKCζ (C24E6) Rabbit mAb              | 9368      | 40 µl    | W               | H, M, R, Mk              | 78 kDa   | Rabbit |
| PKD/PKCμ Antibody                    | 2052      | 40 µl    | W, F            | H, M, R, Mk              | 115 kDa  | Rabbit |
| Anti-rabbit IgG, HRP-linked Antibody | 7074      | 100 µl   |                 |                          |          | Goat   |

**Description:** PKC Antibody Sampler Kit contains reagents to examine the total protein levels of various PKC isoforms. The kit contains enough primary and secondary antibodies to perform four Western mini-blot per primary antibody.

**Background:** Activation of PKC is one of the earliest events in a cascade leading to a variety of cellular responses such as secretion, gene expression, proliferation and muscle contraction (1,2). PKC isoforms have been classified into three groups: classical PKCs, which are calcium-dependent via their C2 domains and are activated by phosphatidylserine (PS), diacylglycerol (DAG) and phorbol esters (TPA or PMA) through their cysteine-rich C1 domains, novel PKCs, which are calcium-independent but are still regulated by PS, DAG and TPA and atypical PKCs, which are calcium-independent and do not require PS, DAG or TPA for their activation (3-7). Members of these three PKC groups contain a pseudo-substrate or autoinhibitory domain that binds to the substrate binding site in the catalytic domain, preventing its activation in the absence of cofactors or activators.

Other members have been recently added to the PKC superfamily based on homology within the catalytic domain. PKC, or PKD, is regulated by DAG and TPA through its C1 domain. However, PKD is distinguished by a PH domain, as well as by its unique substrate recognition and Golgi localization. The PKC-related kinases, or PRKs, lack a C1 domain and do not respond to DAG or phorbol esters. Instead, they can be activated by phosphatidylinositol lipids and their kinase activity is directly regulated by small GTPases of the Rho family through Rho binding to the homology region 1 (HR1).

The activity of PKC is under the control of three distinct phosphorylation events. Specifically, Thr500 in the activation loop, the Thr641 autophosphorylation site and the Ser660 hydrophobic site at the carboxy terminus of PKCβ II are phosphorylated *in vivo* (2). For the atypical PKC isoforms, there is no phosphorylation in the hydrophobic



Western blot analysis of extracts from NIH/3T3 cells, untreated, PDGF-treated (50 ng/ml) or TPA-treated (0.2 µM), using PKD/PKCμ Antibody #2052.

region, which has a glutamic acid residue in place of the serine or threonine residue found in other PKC isoforms. The enzyme PDK1, or a close relative, is responsible for PKC activation.

**Specificity/Sensitivity:** All antibodies in this kit detect endogenous levels of total protein from their respective targets. All antibodies only recognize their specified isoform and do not cross-react with other PKC isoforms.

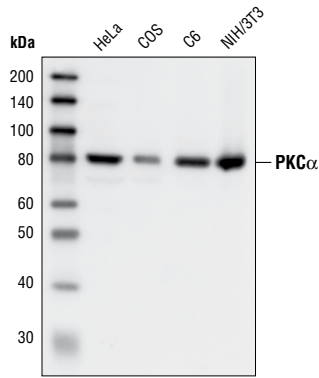
**Source/Purification:** Monoclonal antibody is produced by immunizing rabbits with a synthetic peptide (KLH-coupled) corresponding to human PKCζ.

Polyclonal antibodies are produced by immunizing rabbits with synthetic peptides (KLH-coupled) derived from the sequence of the human protein PKCα, PKCδ, and mouse protein PKD (PKCμ). Antibodies are purified by protein A and peptide affinity chromatography.

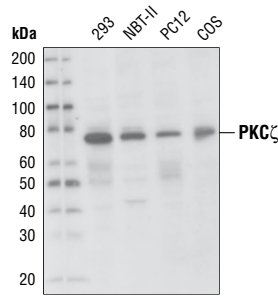
**Storage:** Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at -20°C. Do not aliquot the antibodies.

**Recommended Antibody Dilutions:**  
 Western blotting 1:1000  
 See www.cellsignal.com for individual component dilutions and additional application protocols.

- Companion Products:**
- Phospho-PKD/PKCμ (Ser916) Antibody #2051
  - Phospho-PKD/PKCμ (Ser744/748) Antibody #2054
  - Phospho-PKCδ (Tyr311) Antibody #2055
  - PKCθ Antibody #2059
  - Phospho-PKC (pan) (ζ Thr410) (190D10) Rabbit mAb #2060
  - Phospho-PKC (pan) (βII Ser660) Antibody #9371
  - PKCζ Antibody #9372
  - Phospho-PKCδ (Thr505) Antibody #9374
  - Phospho-PKCα/β II (Thr638/641) Antibody #9375
  - Phospho-PKCδ/θ (Ser643/676) Antibody #9376
  - Phospho-PKCθ (Thr538) Antibody #9377
  - Phospho-PKCζ/λ (Thr410/403) Antibody #9378
  - Phospho-PKC (pan) (γ Thr514) Antibody #9379
  - Anti-rabbit IgG, HRP-linked Antibody #7074
  - Anti-biotin, HRP-linked Antibody #7075
  - Prestained Protein Marker, Broad Range (Premixed Format) #7720
  - Biotinylated Protein Ladder #7727
  - 20X LumiGLO® Reagent and 20X Peroxide #7003
  - Phototope®-HRP Western Blot Detection System, Anti-rabbit IgG, HRP-linked Antibody #7071



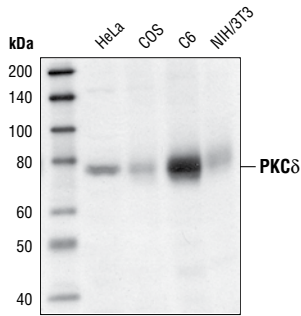
Western blot analysis of extracts of HeLa, COS, C6 and NIH/3T3 cells using **PKCα Antibody #2056**.



Western blot analysis of extracts from 293, NBT-II, PC12 and COS cells using **PKCζ (C24E6) Rabbit mAb #9368**.

**Background References:**

- (1) Nishizuka, Y. (1984) *Nature* 308, 693–698.
- (2) Keranen, L.M. et al. (1995) *Curr. Biol.* 5, 1394–1403.
- (3) Newton, A.C. (1995) *J. Biol. Chem.* 270, 28495–28498.
- (4) Mellor, H. and Parker, P.J. (1998) *Biochem J.* 332 (Pt 2), 281–292.
- (5) Ron, D. and Kazanietz, M.G. (1999) *FASEB J.* 13, 1658–1676.
- (6) Way, K.J. et al. (2000) *Trends Pharmacol. Sci.* 21, 181–187.
- (7) Moscat, J. and Diaz-Meco, M.T. (2000) *EMBO Rep.* 1, 399–403.



Western blot analysis of extracts of HeLa, COS, C6 and NIH/3T3 cells using **PKCδ Antibody #2058**.

## 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<sup>®</sup>-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<sup>®</sup> 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 cm<sup>2</sup>) 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<sup>®</sup> (0.5 ml 20X LumiGLO<sup>®</sup>, 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

**NOTE:** LumiGLO<sup>®</sup> 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<sup>®</sup> incubation and declines over the following 2 hours.