

# PTEN and PDK1 Antibody Sampler Kit

✓ 1 Kit  
(5 x 40 µl)

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This product is for *in vitro* research use only and is not intended for use in humans or animals.  
This product is not intended for use as a therapeutic or in diagnostic procedures.

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Phospho-PTEN (Ser380/Thr382/383) (44A7) Rabbit mAb	9549	40 µl	54 kDa	Rabbit IgG
Non-Phospho PTEN (Ser380/Thr382/Thr383) Antibody	9569	40 µl	54 kDa	Rabbit IgG
PTEN (D4.3) XP™ Rabbit mAb	9188	40 µl	55 kDa	Rabbit IgG
Phospho-PDK1 (Ser241) (C49H2) Rabbit mAb	3438	40 µl	58-68 kDa	Rabbit IgG
PDK1 Antibody	3062	40 µl	58-68 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

See [www.cellsignal.com](http://www.cellsignal.com) for individual component applications, species cross-reactivity, dilutions and additional application protocols.

**Description:** The PTEN and PDK1 Sampler Kit provides an economical means to evaluate two key enzymes that regulate multiple signaling pathways. The kit contains enough primary and secondary antibodies to perform four western mini-blot per primary antibody.

**Background:** PTEN (phosphatase and tensin homologue deleted on chromosome ten), also referred to as MMAC (mutated in multiple advanced cancers) phosphatase, is a tumor suppressor implicated in a wide variety of human cancers (1). PTEN encodes a 403 amino acid polypeptide originally described as a dual-specificity protein phosphatase (2). The main substrates of PTEN are inositol phospholipids generated by the activation of the phosphoinositide 3-kinase (PI3K) (3). PTEN is a major negative regulator of the PI3K/Akt signaling pathway (1,4,5). PTEN possesses a carboxy-terminal, noncatalytic regulatory domain with three phosphorylation sites (Ser380, Thr382 and Thr383) that regulate PTEN stability and may affect its biological activity (6,7). PTEN regulates p53 protein levels and activity (8) and is involved in G protein coupled signaling during chemotaxis (9,10).

Phosphoinositide-dependent protein kinase 1 (PDK1) plays a central role in many signal transduction pathways (11,12) including the activation of Akt and the PKC isoenzymes p70 S6 kinase and RSK (13). Through its effects on these kinases, PDK1 is involved in the regulation of a wide variety of processes, including cell proliferation, differentiation and apoptosis.

**Specificity/Sensitivity:** Each antibody in the PTEN and PDK1 Antibody Sampler Kit detects endogenous levels of its target protein. Activation state antibodies detect only target proteins phosphorylated at indicated residues. Non-Phospho PTEN (Ser380/Thr382/Thr383) Antibody detects endogenous levels of PTEN only when dephosphorylated at Ser380, Thr382 and Thr383.

**Source/Purification:** Phospho-specific rabbit monoclonal antibodies are produced by immunizing animals with synthetic phosphopeptides (KLH-coupled) corresponding to residues around Ser380, Thr382 and Thr383 of human PTEN and around Ser241 of human PDK1. PTEN (D4.3) XP™ Rabbit mAb is produced by immunizing animals with a synthetic peptide (KLH-coupled) corresponding to residues in the carboxy-terminal sequence of human PTEN. Polyclonal antibodies are produced by immunizing animals with synthetic peptides (KLH-coupled) corresponding to residues surrounding Ser380/Thr382/Thr383 of human PTEN and surrounding the carboxy terminus of human PDK1. Polyclonal antibodies are purified using protein A and peptide affinity chromatography.

#### Background References:

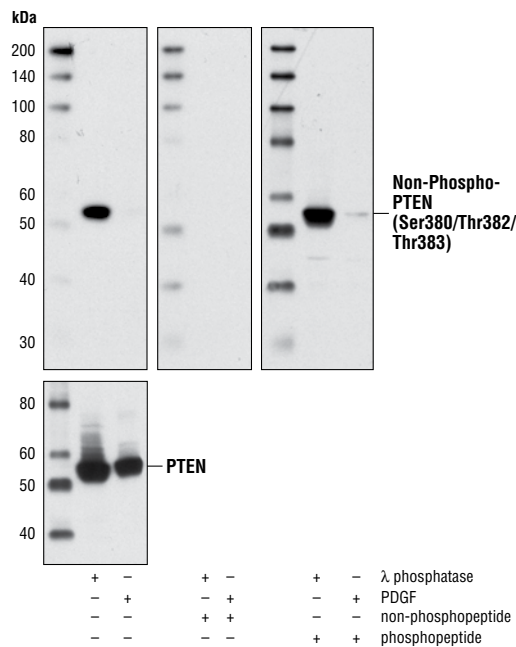
- (1) Cantley, L.C. and Neel, B.G. (1999) *Proc. Natl. Acad. Sci. USA* 96, 4240–4245.
- (2) Myers, M.P. et al. (1997) *Proc. Natl. Acad. Sci. USA* 94, 9052–9057.
- (3) Myers, M.P. et al. (1998) *Proc. Natl. Acad. Sci. USA* 95, 13513–13518.
- (4) Wan, X. and Helman, L.J. (2003) *Oncogene* 22, 8205–8211.
- (5) Wu, X. et al. (1998) *Proc. Natl. Acad. Sci. USA* 95, 15587–15591.
- (6) Vazquez, F. et al. (2000) *Mol. Cell. Biol.* 20, 5010–5018.
- (7) Torres, J. and Pulido, R. (2001) *J. Biol. Chem.* 276, 993–998.
- (8) Freeman, D.J. et al. (2003) *Cancer Cell* 3, 117–130.
- (9) Funamoto, S. et al. (2002) *Cell* 109, 611–623.
- (10) Iijima, M. and Devreotes, P. (2002) *Cell* 109, 599–610.
- (11) Belham, C. et al. (1999) *Curr Biol* 9, R93–6.
- (12) Toker, A. and Newton, A.C. (2000) *Cell* 103, 185–8.
- (13) Williams, M.R. et al. (2000) *Curr Biol* 10, 439–48.

**Storage:** Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at -20°C. *Do not aliquot the antibodies.*

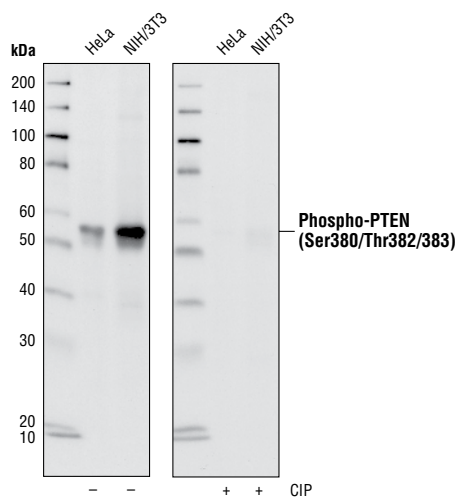
#### Recommended Antibody Dilutions:

Western blotting 1:1000

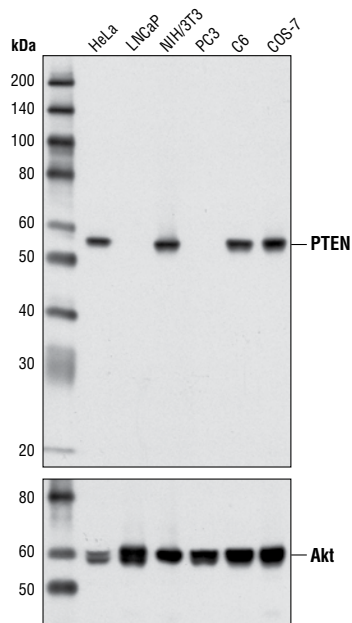
Please visit [www.cellsignal.com](http://www.cellsignal.com) for a complete listing of recommended companion products.



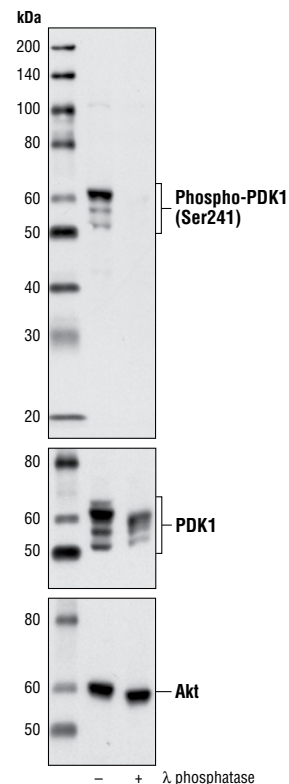
Western blot analysis of extracts from NIH/3T3 cells treated with  $\lambda$  phosphatase or PDGF #9909 using **Non-Phospho-PTEN (Ser380/Thr382/Thr383) Antibody #9569** (upper) or **PTEN (138G6) Rabbit mAb #9559** (lower). The non-phospho-specificity of the antibody was verified by preincubating the antibody with no peptide, with PTEN (Ser380/Thr382/Thr383) non-phosphopeptide or with PTEN (Ser380/Thr382/Thr383) phosphopeptide prior to incubating the membrane.



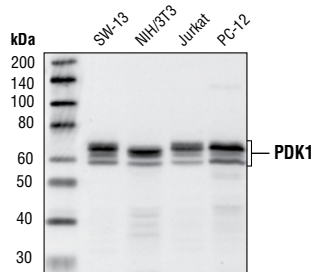
Western blot analysis of extracts from HeLa and NIH/3T3 cells using **Phospho-PTEN (Ser380/Thr382/383) (44A7) Rabbit mAb #9549**. Membranes were left untreated (-) or treated with (+) calf intestinal phosphatase (CIP) following western transfer to verify phospho-specificity of the antibody.



Western blot analysis of extracts from various cell lines using **PTEN (D4.3) XP™ Rabbit mAb #9188** (upper) and **Akt (pan) (C67E7) Rabbit mAb #4691** (lower).



Western blot analysis of extracts from PC3 cells, untreated or  $\lambda$  phosphatase-treated, using **Phospho-PDK1 (Ser241) (C49H2) Rabbit mAb #3438** (upper), **PDK1 Antibody #3062** (middle) or **Akt Antibody #9272** (lower).



Western blot analysis of extracts from SW-13, NIH/3T3, Jurkat and PC12 cells using **PDK1 Antibody #3062**.

## 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.