Store at -20C

#3860

PLCγ Antibody Sampler Kit

☑ 1 Kit
(5 x 20 µl)

Cell Signaling TECHNOLOGY®

Orders ■ 877-616-CELL (2355)
orders@cellsignal.com
Support ■ 877-678-TECH (8324)
info@cellsignal.com
Web ■ www.cellsignal.com

rev. 02/17/17

For Research Use Only. Not For Use In Diagnostic Procedures.

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Phospho-PLCγ1 (Tyr783) (D6M9S) Rabbit mAb	14008	20 µl	155 kDa	Rabbit IgG
PLCγ1 (D9H10) XP® Rabbit mAb	5690	20 µl	150 kDa	Rabbit IgG
Phospho-PLCγ2 (Tyr1217) Antibody	3871	20 µl	150 kDa	Rabbit IgG
PLCγ2 (E5U4T) Rabbit mAb	55512	20 µl	150 kDa	Rabbit IgG
Phospho-PLCγ2 (Tyr759) (E9E9Y) Rabbit mAb	50535	20 µl	150 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: PLCγ Antibody Sampler Kit provides an economical means of analyzing phospho and total PLCγ levels. PLCγ Antibody Sampler Kit includes enough antibody to perform two western blot experiments with each primary antibody.

Background: Phosphoinositide-specific phospholipase C (PLC) plays a significant role in transmembrane signaling. In response to extracellular stimuli such as hormones, growth factors, and neurotransmitters, PLC hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP_2) to generate two secondary messengers: inositol 1,4,5-triphosphate (IP_3) and diacylglycerol (DAG) (1). At least four families of PLCs have been identified: PLCβ, PLCγ, PLCδ, and PLCε. Phosphorylation is one of the key mechanisms that regulate the activity of PLC. PLCγ is activated by both receptor and non-receptor tyrosine kinases (2). PLCγ forms a complex with EGF and PDGF receptors, which leads to the phosphorylation of PLCγ at Tyr771, 783, and 1248 (3). Phosphorylation by Syk at Tyr783 activates the enzymatic activity of PLCγ1 (4). PLCγ2 is engaged in antigen-dependent signaling in B cells and collagen-dependent signaling in platelets. Phosphorylation by Btk or Lck at Tyr753, 759, 1197, and 1217 is correlated with PLCγ2 activity (5,6).

Specificity/Sensitivity: Each antibody in the PLCγ Antibody Sampler Kit detects endogenous levels of its target protein. The antibodies do not cross react with other PLCs.

Source/Purification: Monoclonal phospho-specific antibodies are produced by immunizing animals with a synthetic phospho-peptide corresponding to residues surrounding Leu1220 of human PLCγ1 protein, Tyr759 of human PLCγ2 protein, or Tyr783 of PLCγ1. Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues near the amino terminus of human PLCγ2 protein.

Polyclonal antibodies are produced by immunizing animals with a synthetic phospho-peptide corresponding to residues surrounding Tyr1217 of human PLCγ2. Antibodies are purified by protein A and peptide affinity chromatography..

Background References:

(1) Singer, W.D. et al. (1997) *Annu Rev Biochem* 66, 475-509.

(2) Margolis, B. et al. (1989) *Cell* 57, 1101-7.

(3) Kim, H.K. et al. (1991) *Cell* 65, 435-41.

(4) Wang, Z. et al. (1998) *Mol Cell Biol* 18, 590-7.

(5) Watanabe, D. et al. (2001) *J Biol Chem* 276, 38595-601.

(6) Ozdener, F. et al. (2002) *Mol Pharmacol* 62, 672-9.

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 validation data and a complete listing of recommended companion products.

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**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

#3860

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.