#8350 Store at -20°C

Phospho-TSC2 Antibody Sampler Kit

☑ 1 Kit
(4 x 20 µl)

Cell Signaling TECHNOLOGY®

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

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Tuberin/TSC2 (D93F12) XP® Rabbit mAb	4308	20 µl	200 kDa	Rabbit IgG
Phospho-Tuberin/TSC2 (Ser939) Antibody	3615	20 µl	200 kDa	Rabbit IgG
Phospho-Tuberin/TSC2 (Thr1462) (5B12) Rabbit mAb	3617	20 µl	200 kDa	Rabbit IgG
Phospho-Tuberin/TSC2 (Ser1387) Antibody	5584	20 µl	200 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.

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 for validation data and a complete listing of recommended companion products.

U.S. Patent No. 5,675,063

Tween®20 is a registered trademark of ICI Americas, Inc.

Description: The Phospho-TSC2 Antibody Sampler Kit provides an economical means to investigate protein folding within the cell. The kit includes enough antibody to perform two western blot experiments with each primary antibody.

Background: Tuberin is a product of the TSC2 tumor suppressor gene and an important regulator of cell proliferation and tumor development (1). Mutations in either *TSC2* or the related *TSC1* (hamartin) gene cause tuberous sclerosis complex (TSC), an autosomal dominant disorder characterized by development of multiple, widespread non-malignant tumors (2). Tuberin is directly phosphorylated at Thr1462 by Akt/PKB (3). Phosphorylation at Thr1462 and Tyr1571 regulates tuberin-hamartin complexes and tuberin activity (3-5). In addition, tuberin inhibits the mammalian target of rapamycin (mTOR), which promotes inhibition of p70 S6 kinase, activation of eukaryotic initiation factor 4E binding protein 1 (4E-BP1, an inhibitor of translation initiation), and eventual inhibition of translation (3,6,7).

Specificity/Sensitivity: Each antibody in the Phospho-TSC2 Antibody Sampler Kit recognizes only its specific target and does not cross-react with other family members unless otherwise indicated. Phospho-Tuberin/TSC2 (Ser1387) Antibody detects endogenous levels of tuberin protein only when phosphorylated at Ser1387. This antibody also cross-reacts with an unidentified 140 kDa protein.

Source/Purification: Monoclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to carboxy-terminal residues of human tuberin protein or a synthetic phosphopeptide corresponding to residues surrounding Thr1462 of human tuberin protein. Polyclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues around Ser939 or corresponding to residues surrounding Ser1387 of human tuberin protein. Polyclonal antibodies are purified by protein A and peptide affinity chromatography.

Background References:

(1) Soucek, T. et al. (1998) *Proc. Natl. Acad. Sci. USA* 95, 15653-15658.

(2) Sparagana, S.P. and Roach, E.S. (2000) *Curr. Opin. Neurol.* 13, 115-119.

(3) Manning, B. D. et al. (2002) *Mol. Cell* 10, 151-161.

(4) Aicher, L. D. et al. (2001) *J. Biol. Chem.* 276, 21017-21021.

(5) Dan, H. C. et al. (2002) *J. Biol. Chem.* 277, 35364-35370.

(6) Goncharova, E.A. et al. (2002) *J. Biol. Chem.* 277, 30958-30967.

(7) Inoki, K. et al. (2002) *Nat. Cell Biol.* 4, 648-657.

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 **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

#8350

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.