Store at -20°C

Phospho-YAP/TAZ Antibody Sampler Kit

#52420

☑ 1 Kit
(7 x 20 µl)

New 04/18

Support: +1-978-867-2388 (U.S.)
www.cellsignal.com/support

Orders: 877-616-2355 (U.S.)
orders@cellsignal.com

Entrez-Gene ID #25937, 10413
UniProt ID #Q9GZV5, P46937

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

Products Included	Product #	Quantity	Mol. Wt.	Isotype/Source
P-YAP (S109) Rabbit Ab	46931	20 µl	78 kDa	Rabbit
P-YAP (S127) (D9W2I) Rabbit mAb	13008	20 µl	65-75 kDa	Rabbit IgG
P-YAP (S397) (D1E7Y) Rabbit mAb	13619	20 µl	75 kDa	Rabbit IgG
P-TAZ (S89) (E1X9C) Rabbit mAb	59971	20 µl	55 kDa	Rabbit IgG
YAP/TAZ (D24E4) Rabbit mAb	8418	20 µl	50, 70 kDa	Rabbit IgG
YAP (D8H1X) XP® Rabbit mAb	14074	20 µl	65-75 kDa	Rabbit IgG
TAZ (D3I6D) Rabbit mAb	70148	20 µl	50 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: The Phospho-YAP/TAZ Antibody Sampler Kit uses phospho-specific and control antibodies to provide an economical means of detecting the phosphorylation of YAP and TAZ proteins at critical residues that are reported to regulate YAP and TAZ protein stability. The kit includes enough antibody to perform two western blot experiments with each primary antibody.

Background: YAP and TAZ (WWTR1) are transcriptional co-activators that play a central role in the Hippo Signaling pathway that regulates cell, tissue and organ growth. YAP and TAZ are structurally and functionally similar, but exhibit differential patterns of expression among cells and tissues that suggest partially non-redundant functions (1). YAP and TAZ are dynamically regulated in response to internal and external cellular signals. Under growth conditions, YAP and TAZ are localized in the nucleus, where they interact with transcription factors (e.g., TEA domain proteins) that regulate the transcription of genes that control proliferation and cell survival (2). The subcellular localization of YAP and TAZ is dynamically regulated by a kinase cascade that regulates the phosphorylation status of key residues within YAP and TAZ. Phosphorylation of YAP and TAZ (e.g., Ser109, Ser127, Ser397 in YAP; Ser89 in TAZ) results in their cytoplasmic translocation, sequestration by 14-3-3 proteins, and recruitment of the β-TrCP (SCF) ubiquitin ligase complex (3,4). This complex ubiquitinates YAP and TAZ, triggering their proteolytic degradation in the proteasome, thereby altering the transcription of genes that control proliferation and cell survival (3-5).

Specificity/Sensitivity: Each antibody in the Phospho-YAP/TAZ Antibody Sampler Kit detects endogenous levels of its target protein. Phospho-YAP (Ser127) (D9W2I) Rabbit mAb recognizes endogenous levels of YAP protein only when phosphorylated at Ser127. Phospho-YAP (Ser397) (D1E7Y) Rabbit mAb recognizes endogenous levels of YAP protein only when phosphorylated at Ser397. Phospho-YAP (Ser109) Antibody recognizes endogenous levels of YAP protein only when phosphorylated at Ser109. Phospho-TAZ (Ser89) (E1X9C) Rabbit mAb recognizes endogenous levels of TAZ protein only when phosphorylated at Ser89. Due to epitope sequence similarities, Phospho-TAZ (Ser89) (E1X9C) Rabbit mAb may weakly detect YAP protein, but only when YAP is phosphorylated at Ser127.

Source/Purification: Monoclonal antibodies are produced by immunizing rabbits with recombinant protein corresponding to the carboxy terminus of human YAP protein and synthetic peptides corresponding to Ala200 of mouse TAZ protein and Asp362 of human TAZ protein. Phosphorylation-specific antibodies are produced by immunizing rabbits with synthetic phospho-peptides corresponding to Ser109, Ser127, and Ser397 of human YAP protein, and Ser89 of human TAZ protein. Polyclonal 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, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. *Do not aliquot the antibody.*

Recommended Antibody Dilutions:

Western blotting	1:1000

For product specific protocols and a complete listing of recommended companion products please see the product web page at www.cellsignal.com.

Background References:

(1) Piccolo, S. et al. (2014) *Physiol Rev* 94, 1287-312.
(2) Holden, J.K. and Cunningham, C.N. (2018) *Cancers (Basel)* 10, .
(3) Lei, Q.Y. et al. (2008) *Mol Cell Biol* 28, 2426-36.
(4) Zhao, B. et al. (2010) *Genes Dev* 24, 72-85.
(5) Cordenonsi, M. et al. (2011) *Cell* 147, 759-72.

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Applications: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide **Species Cross-Reactivity:** **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.

Western Immunoblotting Protocol

For western blots, incubate membrane with diluted primary antibody in either 5% w/v BSA or nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.
NOTE: Please refer to primary antibody datasheet or product webpage for recommended primary antibody dilution buffer and recommended antibody dilution.

A. Solutions and Reagents

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

1. **20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH_2O, mix.
2. **10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH_2O, mix.
3. **1X SDS Sample Buffer:** Blue Loading Pack (#7722) or Red Loading Pack (#7723) Prepare fresh 3X reducing loading buffer by adding 1/10 volume 30X DTT to 1 volume of 3X SDS loading buffer. Dilute to 1X with dH_2O.
4. **10X Tris-Glycine SDS Running Buffer:** (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml dH_2O, mix.
5. **10X Tris-Glycine Transfer Buffer:** (#12539) To prepare 1 L 1X transfer buffer: add 100 ml 10X transfer buffer to 200 ml methanol + 700 ml dH_2O, mix.
6. **10X Tris Buffered Saline with Tween® 20 (TBST):** (#9997) To prepare 1 L 1X TBST: add 100 ml 10X TBST to 900 ml dH_2O, mix.
7. **Nonfat Dry Milk:** (#9999)
8. **Blocking Buffer:** 1X TBST with 5% w/v nonfat dry milk; for 150 ml, add 7.5 g nonfat dry milk to 150 ml 1X TBST and mix well.
9. **Wash Buffer:** (#9997) 1X TBST
10. **Bovine Serum Albumin (BSA):** (#9998)
11. **Primary Antibody Dilution Buffer:** 1X TBST with 5% BSA or 5% nonfat dry milk as indicated on primary antibody datasheet; for 20 ml, add 1.0 g BSA or nonfat dry milk to 20 ml 1X TBST and mix well.
12. **Biotinylated Protein Ladder Detection Pack:** (#7727)
13. **Prestained Protein Marker, Broad Range (Premixed Format):** (#7720)
14. **Blotting Membrane and Paper:** (#12369) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.
15. **Secondary Antibody Conjugated to HRP:** anti-rabbit (#7074); anti-mouse (#7076)
16. **Detection Reagent:** LumiGLO® chemiluminescent reagent and peroxide (#7003) or SignalFire™ ECL Reagent (#6883)

B. Protein Blotting

A general protocol for sample preparation.

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 for a 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 sec to complete cell lysis and shear DNA (to reduce sample viscosity).
5. Heat a 20 µl sample to 95–100°C for 5 min; cool on ice.
6. Microcentrifuge for 5 min.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm). **NOTE:** Loading of prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights are recommended.
8. Electrotransfer to nitrocellulose membrane (#12369).

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.

I. Membrane Blocking

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 min at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hr at room temperature.
3. Wash three times for 5 min each with 15 ml of TBST.

II. Primary Antibody Incubation

1. Incubate membrane and primary antibody (at the appropriate dilution and diluent as recommended in the product datasheet) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
2. Wash three times for 5 min each with 15 ml of TBST.
3. Incubate membrane with the species appropriate HRP-conjugated secondary antibody (#7074 or #7076 at 1:2000) and anti-biotin, HRP-linked Antibody (#7075 at 1:1000–1:3000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hr at room temperature.
4. Wash three times for 5 min each with 15 ml of TBST.
5. Proceed with detection (Section D).

D. Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO® #7003, 0.5 ml 20X peroxide, and 9.0 ml purified water) or 10 ml SignalFire™ #6883 (5 ml Reagent A, 5 ml Reagent B) with gentle agitation for 1 min at room temperature.
2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10 sec exposure should indicate the proper exposure time. **NOTE:** Due to the kinetics of the detection reaction, signal is most intense immediately following incubation and declines over the following 2 hr.