#12747 Store at -20°C

Smad2/3 Antibody Sampler Kit

☑ 1 Kit
(6 x 40 µl)

Cell Signaling TECHNOLOGY®

Orders ■ 877-616-CELL (2355)
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Support ■ 877-678-TECH (8324)
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For Research Use Only. Not For Use In Diagnostic Procedures.

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Phospho-Smad2 (Ser465/467) (138D4) Rabbit mAb	3108	40 µl	60 kDa	Rabbit IgG
Smad2 (D43B4) XP® Rabbit mAb	5339	40 µl	60 kDa	Rabbit IgG
Smad2/3 (D7G7) XP® Rabbit mAb	8685	40 µl	52, 60 kDa	Rabbit IgG
Phospho-Smad3 (Ser423/425) (C25A9) Rabbit mAb	9520	40 µl	52 kDa	Rabbit IgG
Smad3 (C67H9) Rabbit mAb	9523	40 µl	52 kDa	Rabbit IgG
Smad4 Antibody	9515	40 µl	70 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

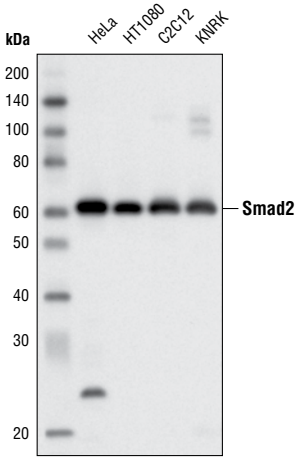
See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.

Description: The Smad2/3 Antibody Sampler Kit provides an economical means of detecting target proteins of the TGF-β signaling pathway. The kit contains enough primary and secondary antibodies to perform four western blots with each antibody.

Background: Transforming growth factor-β (TGF-β) superfamily signaling plays a critical role in the regulation of cell growth, differentiation, and development in a wide range of biological systems. In general, signaling is initiated with ligand-induced oligomerization of serine/ threonine receptor kinases and phosphorylation of the cytoplasmic signaling molecules Smad2 and Smad3 for the TGF-β/ activin pathway, or Smad1/5/8 for the bone morphogenetic protein (BMP) pathway. Carboxy-terminal phosphorylation of Smad proteins by activated receptors results in their partnering with the common signaling transducer Smad4, and translocation to the nucleus. Activated Smad proteins regulate diverse biological effects by partnering with transcription factors resulting in cell-state specific modulation of transcription (1-4).

Specificity/Sensitivity: Each antibody in the Smad2/3 Antibody Sampler Kit recognizes only its specific target and does not cross-react with other family members. Activation state antibodies detect their intended targets only when phosphorylated at the indicated site. The total Smad2, Smad3, and Smad4 antibodies detect their respective targets at endogenous levels.

Source/Purification: Phospho-specific monoclonal antibodies are produced by immunizing animals with synthetic phosphopeptides corresponding to residues surrounding Ser465/467 of human Smad2 and Ser423/425 of human Smad3. Total Smad2 and Smad3 monoclonal antibodies are produced by immunizing animals with synthetic peptides corresponding to residues near the amino termini of mouse Smad2 and Smad3 or His198 of human Smad2/3 protein. Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to the residues surrounding Pro278 of human Smad4. Antibodies are purified by protein A and peptide affinity chromatography.



Western blot analysis of extracts from various cell lines using ***Smad2 (D43B4) XP® Rabbit mAb #5339****.*

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 a complete listing of recommended companion products.

Background References:

(1) Horbelt, D. et al. (2012) *Int J Biochem Cell Biol* 44, 469-74.

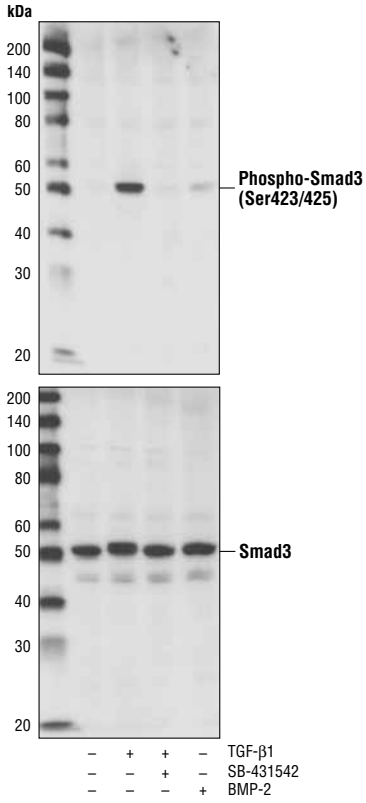
(2) Ikushima, H. and Miyazono, K. (2010) *Nat Rev Cancer* 10, 415-24.

(3) Kitisin, K. et al. (2007) *Sci STKE* 2007, cm1.

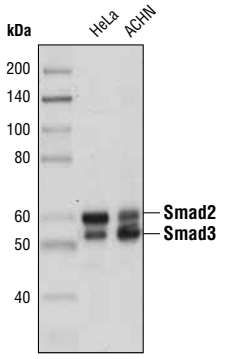
(4) Schmierer, B. and Hill, C.S. (2007) *Nat Rev Mol Cell Biol* 8, 970-82.

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

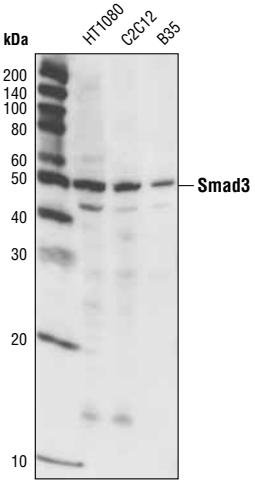
Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.



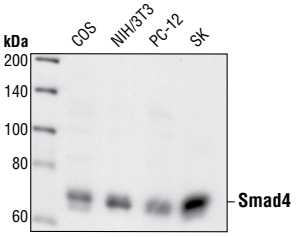
Western blot analysis of extracts from HT-1080 cells, untreated (-) or treated with TGF-β1, TGFR inhibitor SB-431542 or BMP-2 (+)-2, using ***Phospho-Smad3 (Ser423/425) (C25A9) Rabbit mAb #9520*** *(top) or total* ***Smad3 (C67H9) Rabbit mAb #9523*** *(bottom).*



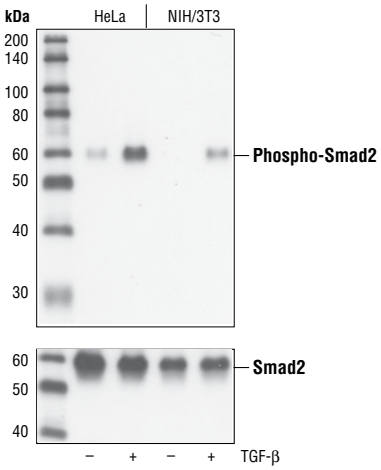
Western blot analysis of extracts from HeLa and ACHN cells using ***Smad2/3 (D7G7) XP® Rabbit mAb #8685****.*



Western blot analysis of extracts from HT1080 (human), C2C12 (mouse) and B35 (rat) cells using ***Smad3 (C67H9) Rabbit mAb #9523****.*



Western blot analysis of extracts from various cell lines using ***Smad4 Antibody #9515****.*



Western blot analysis of extracts from untreated (-) or TGF-β treated (+) HeLa and NIH/3T3 cells, using ***Phospho-Smad2 (Ser465/467) (138D4) Rabbit mAb #3108*** *(upper) or Smad2 Antibody #3102 (lower).*

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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.