

#9519 Store at -20°C

# Smad6 Antibody

✓ 100 µl  
(10 Western mini-blot)

**Orders** ■ 877-616-CELL (2355)  
orders@cellsignal.com  
**Support** ■ 877-678-TECH (8324)  
info@cellsignal.com  
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New 07/06

This product is for *in vitro* research use only and is not intended for use in humans or animals.

Applications	Species Cross-Reactivity	Molecular Wt.	Source
W	H, M	62 kDa	Rabbit

**Background:** Members of the Smad family of signal transduction molecules are components of a critical intracellular pathway that transmits TGF-β signals from the cell surface into the nucleus. Three distinct classes of Smads have been defined: the receptor-regulated Smads (R-Smads), which include Smad1, 2, 3, 5 and 8, the common-mediator Smad (co-Smad), Smad4, and the antagonistic or inhibitory Smads (I-Smads), Smad6 and 7 (1-5). Briefly, activated type I receptors associate with specific R-Smads and phosphorylate them on a conserved carboxy-terminal SSXS motif. The phosphorylated R-Smad dissociates from the receptor and forms a heteromeric complex with the co-Smad (Smad4), allowing translocation of the complex to the nucleus. Once in the nucleus, Smads can target a variety of DNA binding proteins to regulate transcriptional responses (6-8).

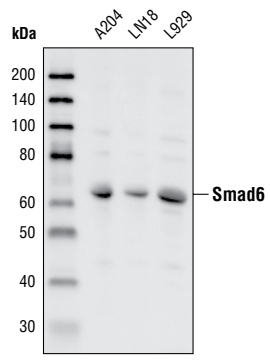
The inhibitory Smads, Smad6 and Smad7, were originally identified in vascular endothelium and inhibit TGF-β and BMP signaling by interfering with the phosphorylation of other Smad family members (9,10). Their expression may be induced by TGF-β, and BMP in some cell types providing a negative feedback loop (11,12).

**Specificity/Sensitivity:** Smad6 Antibody detects endogenous levels of total Smad6 protein.

**Source/Purification:** Polyclonal antibodies are produced by immunizing rabbits with a synthetic peptide (KLH-coupled) corresponding to residues surrounding cysteine 55 of human Smad6. Antibodies were purified by protein A and peptide affinity chromatography.

### Background References:

- (1) Heldin, C. H. et al. (1997) *Nature* 390, 465-471.
- (2) Attisano, L. and Wrana, J.L. (1998) *Curr. Opin. Cell Biol.* 10, 188-194.
- (3) Derynck, R. et al. (1998) *Cell* 95, 737-740.
- (4) Massague, J. (1998) *Annu. Rev. Biochem.* 67, 753-791.



Western blot analysis of extracts from A204, LN18 and L929 cells, using Smad6 Antibody.

- (5) Whitman, M. et al. (1998) *Genes Dev.* 12, 2445-2462.
- (6) Wrana, J. (2000) *Science* 23, 1-9.
- (7) Attisano, L. and Wrana, J. (2002) *Science* 296, 1646-1647.
- (8) Moustakas, A. et al. (2001) *J. Cell Sci.* 114, 4359-4369.
- (9) Topper, J.N. et al. (1997) *Proc. Natl. Acad. Sci. USA* 94, 9314-9319.
- (10) Imamura, T. et al. (1997) *Nature* 389, 622-626.
- (11) Takase, M. et al. (1998) *Biochem. Biophys. Res. Commun.* 244, 26-29.
- (12) Afrakhte, M. et al. (1998) *Biochem. Biophys. Res. Commun.* 249, 505-511.

**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 antibody.

**Recommended Antibody Dilutions:**  
Western blotting 1:1000

### Companion Products:

- Phospho-Smad1 (Ser463/465)/ Smad5 (Ser463/465)/ Smad8 (Ser426/428) Antibody #9511
- Smad1 Antibody #9512
- Phospho-Smad2 (Ser465/467) Antibody #3101
- Phospho-Smad2 (Ser465/467) (138D4) Rabbit mAb #3108
- Smad2 (L16D3) Mouse mAb #3103
- Smad2/3 Antibody #3102
- Phospho-Smad3 (Ser423/425)/Smad1 (Ser463/465) Antibody #9514
- Smad4 Antibody #9515
- Smad5 Antibody #9517
- Phototope®-HRP Western Blot Detection System, Anti-rabbit IgG, HRP-linked Antibody #7071
- Anti-rabbit IgG, HRP-linked Antibody #7074
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder Detection Pack #7727
- 20X LumiGLO® Reagent and 20X Peroxide #7003

**IMPORTANT: 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.**

**Applications Key:** W—Western IP—Immunoprecipitation IHC—Immunohistochemistry IC—Immunocytochemistry IF—Immunofluorescence F—Flow cytometry E—ELISA D—DELFIA®  
**Species Cross-Reactivity Key:** H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken X—Xenopus Z—zebra fish B—bovine All—all species expected  
Species enclosed in parentheses are predicted to react based on 100% sequence homology.

## Western Immunoblotting Protocol (Primary Ab 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.