Store at -20°C

#8358

# Hedgehog Signaling 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

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

| Products Included | Product # | Quantity | Mol. Wt. | Isotype |
|---|---|---|---|---|
| Shh (C9C5) Rabbit mAb | 2207 | 20 µl | 19, 45 kDa | Rabbit IgG |
| PTCH1 (C53A3) Rabbit mAb | 2468 | 20 µl | 180-210 kDa | Rabbit IgG |
| PTCH2 (G1191) Antibody | 2470 | 20 µl | 130 kDa | Rabbit IgG |
| SUFU (C54G2) Rabbit mAb | 2520 | 20 µl | 54 kDa | Rabbit IgG |
| GLI1 (C68H3) Rabbit mAb | 3538 | 20 µl | 160 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.**

**Description:** This sampler kit provides an economical means of evaluating key members of the Hedgehog signaling pathway. The kit includes enough antibody to perform two western blot experiments for each primary antibody.

**Background:** The evolutionarily conserved Hedgehog (Hh) signaling pathway plays critical roles in the regulation of patterning, growth, and cell migration during embryonic development and adult tissue homeostasis. Aberrant Hh signaling activity can be associated with numerous birth defects and uncontrolled Hh pathway activation is linked to the development of several types of cancers (1-2). The three identified vertebrate Hh genes are Sonic (Shh), Indian (Ihh), and Desert (Dhh), all of which have distinct as well as overlapping roles (3-5). Patched1 and 2 (PTCH1 and PTCH2) are twelve-pass transmembrane proteins that function as the Hh receptors (6-9). The general organization of the Hh pathway consists of a series of repressive interactions. In the absence of Hh proteins (off-state), PTCH suppresses the otherwise constitutively active signaling receptor Smoothened (Smo) (1,2). In the off-state, SUFU (Suppressor of Fused), originally identified in *Drosophila as* a suppressor of the Fused (Fu) kinase (10), suppresses Hh signaling by regulating the localization of the transcription factors Gli and Ci (11,12). In *Drosophila*, SUFU may also positively regulate Hh signaling depending on SUFU protein levels and Hh signal intensity (13).

**Specificity/Sensitivity:** Shh (C9C5) Rabbit mAb detects endogenous levels of total Shh protein. This antibody does not cross-react with transfected IHH and DHH. PTCH1 (C53A3) Rabbit mAb detects transfected levels of PTCH1. This antibody can also detect endogenous levels of PTCH1 through immunoprecipitation followed by Western blot analysis. PTCH2 (G1191) Antibody detects transfected levels of PTCH2 protein. It does not recognize transfected levels of human PTCH1 protein. SUFU (C54G2) Rabbit mAb detects endogenous levels of total SUFU protein. GLI1 (C68H3) Rabbit mAb detects endogenous levels of total GLI1 protein.

**Source/Purification:** Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to a region (predicted to be intracellular) surrounding Gly1191 of human PTCH2. Polyclonal antibodies are purified by protein A and peptide affinity chromatography. Monoclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Glu53 of human Shh, Pro1307 of human PTCH1, Leu458 of human SUFU, or Gly420 of human GLI1.

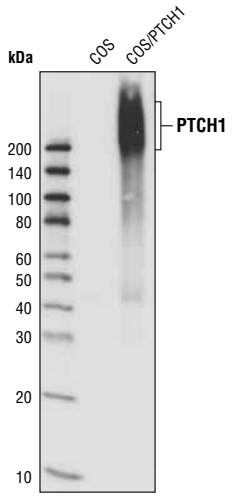
**Background References:**

(1) Ingham, P.W. and McMahon, A.P. (2001) *Genes Dev* 15, 3059-87.
(2) McMahon, A.P. et al. (2003) *Curr Top Dev Biol* 53, 1-114.
(3) Zhang, X.M. et al. (2001) *Cell* 106, 781-92.
(4) Adolphe, C. et al. (2004) *Development* 131, 5009-19.
(5) Pathi, S. et al. (2001) *Mech Dev* 106, 107-17.
(6) Stone, D.M. et al. (1996) *Nature* 384, 129-34.
(7) Chen, Y. and Struhl, G. (1996) *Cell* 87, 553-63.
(8) Motoyama, J. et al. (1998) *Nat Genet* 18, 104-6.
(9) Smyth, I. et al. (1999) *Hum Mol Genet* 8, 291-7.
(10) Pham, A. et al. (1995) *Genetics* 140, 587-98.
(11) Barnfield, P.C. et al. (2005) *Differentiation* 73, 397-405.
(12) Méthot, N. and Basler, K. (2000) *Development* 127, 4001-10.
(13) Dussillol-Godar, F. et al. (2006) *Dev Biol* 291, 53-66.

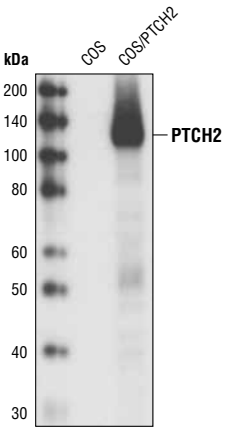


**Applications Key:** **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide

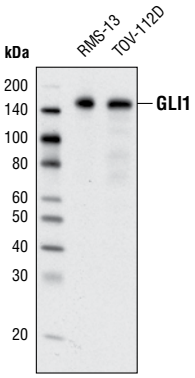
**Species Cross-Reactivity Key:** **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **All**—all species expected Species enclosed in parentheses are predicted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



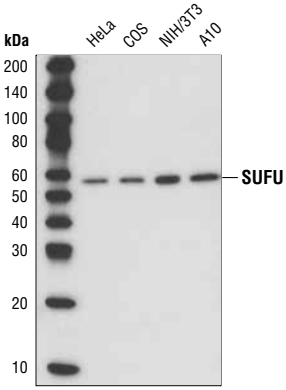

*Western blot analysis of total cell lysates from COS cells, untransfected or transiently transfected with a human PTCH1 expression construct, using* ***PTCH1 (C53A3) Rabbit mAb #2468****.*




*Western blot analysis of extracts from COS cells, untransfected or transiently transfected with a construct expressing human PTCH2, using* ***PTCH2 (G1191) Antibody #2470****.*




*Western blot analysis of extracts from RMS-13 and TOV-112D cells using* ***GLI1 (C68H3) Rabbit mAb #3538****.*




*Western blot analysis of total cell lysates from various cell types using* ***SUFU (C54G2) Rabbit mAb #2520****.*

#8358

# 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.