

# Jak/Stat Pathway Inhibitors Antibody Sampler Kit

1 Kit  
(6 x 20 µl)



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

Products Included	Product #	Quantity	Mol. Wt.	Isotype
PIAS1 (D33A7) XP® Rabbit mAb	3550	20 µl	76 kDa	Rabbit IgG
PIAS3 (D5F9) XP® Rabbit mAb	9042	20 µl	65-75 kDa	Rabbit IgG
PIAS4 (D2F12) Rabbit mAb	4392	20 µl	75 kDa	Rabbit IgG
SOCS1 (A156) Antibody	3950	20 µl	23 kDa	Rabbit IgG
SOCS2 Antibody	2779	20 µl	22 kDa	Rabbit IgG
SOCS3 (D6E1T) Rabbit mAb	52113	20 µl	26 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

See [www.cellsignal.com](http://www.cellsignal.com) for individual component applications, species cross-reactivity, dilutions, and additional application protocols.

**Description:** The Jak/Stat Pathway Inhibitors Antibody Sampler Kit provides an economical means to examine several inhibitors of Jak/Stat signaling, including PIAS1, PIAS3, PIAS4, SOCS1, SOCS2, and SOCS3. The kit contains enough primary antibody to perform two western blot experiments with each primary antibody.

**Background:** Jak (Janus Kinase) and Stat (signal transducer and activator of transcription) proteins are utilized by receptors for a wide variety of ligands including cytokines, hormones, growth factors, and neurotransmitters (1). Jaks and Stats play important roles in oncogenesis, tumor progression, angiogenesis, cell motility, immune responses, and stem cell differentiation (2-5). Therefore, regulation of Jak/Stat signaling is crucial to prevent aberrant signaling which can lead to disease progression. Two methods for regulating Jak/Stat signaling involve SOCS and PIAS proteins (6,7).

The SOCS (suppressor or cytokine signaling) family members are negative regulators of cytokine signal transduction that inhibit the Jak/Stat pathway and consist of 8 known members, including the originally identified protein CIS1 (cytokine-inducible SH2-containing protein) and SOCS1-SOCS7. Each SOCS family member contains a central SH2 domain and a conserved carboxy-terminal motif designated as the SOCS box. These proteins are important regulators of cytokine signaling, proliferation, differentiation, and immune responses (8-10). SOCS proteins are involved in regulating over 30 cytokines, including interleukins, growth hormone (GH), interferons, leptin, and leukemia inhibitory factor (7). SOCS1, also known as JAB (Janus Kinase binding protein) and SSI-1 (Stat-induced Stat inhibitor-1), shares the most homology with SOCS3 and both are highly induced by cytokines (7,11). Both SOCS1 and SOCS3 directly inhibit Jak activity; SOCS1 inhibits Jak through an interaction involving a phosphotyrosine located in the kinase activation loop; SOCS3 inhibits Jak via its SH2 domain (12,13). In addition to inhibiting Jak/Stat signaling, the SOCS box of SOCS1 and SOCS3 can trigger ubiquitin-mediated degradation of

proteins within and outside the Jak/Stat pathway (14,15). SOCS2 is also induced upon cytokine stimulation and the activity of SOCS2 has been predominately linked to GH and insulin-like growth factor signaling by binding to tyrosine-phosphorylated receptors via its SH2 domain (11,16).

The PIAS (protein inhibitor of activated Stats) proteins, which include PIAS1, PIAS3, PIASx, and PIASy (PIAS4), were originally characterized based on their interaction with the Stat family of transcription factors (16,17). PIAS1, PIAS3, and PIASx interact with and repress Stat1, Stat3, and Stat4, respectively (17-19). The PIAS family contains a conserved RING domain that has been linked to function as a SUMO (small ubiquitin-related modifier) ligase, coupling the SUMO conjugating enzyme Ubc9 with its substrate proteins leading to regulation of transcription factors through distinct mechanisms including NF-κB, c-Jun, Oct-4, p53, and SMADs. PIAS4 is a specific SUMO-E3 ligase for Ets-1 and represses Ets-1 dependent transcription in addition to altering the nuclear localization and reducing the transcriptional activity of C/EBPδ, thereby enhancing cell proliferation and migration (20,21).

**Specificity/Sensitivity:** Each antibody in this kit recognizes only the specific target protein and does not cross-react with other family members.

**Source/Purification:** Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Ala156 of human SOCS1 protein, residues at the carboxy terminus of human SOCS2 protein, or recombinant protein specific to human SOCS3 protein. 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 Ser550 of human PIAS1 protein, Pro590 of human PIAS3 protein, or Lys59 of human PIAS4 protein.

**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](http://www.cellsignal.com) for validation data and a complete listing of recommended companion products.

**Background References:**

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- (12) Yasukawa, H. et al. (1999) *EMBO J* 18, 1309-20.
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- (18) Chung, C.D. et al. (1997) *Science* 278, 1803-5.
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# 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.

- 20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH<sub>2</sub>O, mix.
- 10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH<sub>2</sub>O, mix.
- 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<sub>2</sub>O.
- 10X Tris-Glycine SDS Running Buffer:** (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml dH<sub>2</sub>O, mix.
- 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<sub>2</sub>O, mix.
- 10X Tris Buffered Saline with Tween® 20 (TBST):** (#9997) To prepare 1 L 1X TBST: add 100 ml 10X TBST to 900 ml dH<sub>2</sub>O, mix.
- Nonfat Dry Milk:** (#9999)
- 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.
- Wash Buffer:** (#9997) 1X TBST
- Bovine Serum Albumin (BSA):** (#9998)
- 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.
- Biotinylated Protein Ladder Detection Pack:** (#7727)
- Prestained Protein Marker, Broad Range (Premixed Format):** (#7720)
- Blotting Membrane and Paper:** (#12369) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.
- Secondary Antibody Conjugated to HRP:** anti-rabbit (#7074); anti-mouse (#7076)
- Detection Reagent:** LumiGLO® chemiluminescent reagent and peroxide (#7003) or SignalFire™ ECL Reagent (#6883)

## B. Protein Blotting

**A general protocol for sample preparation.**

- 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 for a 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 sec to complete cell lysis and shear DNA (to reduce sample viscosity).
- Heat a 20 µl sample to 95–100°C for 5 min; cool on ice.
- Microcentrifuge for 5 min.
- 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.
- Electrotransfer to nitrocellulose membrane (#12369).

## 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.

### I. Membrane Blocking

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

### II. Primary Antibody Incubation

- 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.
- Wash three times for 5 min each with 15 ml of TBST.
- 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.
- Wash three times for 5 min each with 15 ml of TBST.
- Proceed with detection (Section D).

## D. Detection of Proteins

- 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.
- 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.