

bs-5235R**[Primary Antibody]****phospho-Smad3 (Ser204) Rabbit pAb****Bioss**
ANTIBODIES

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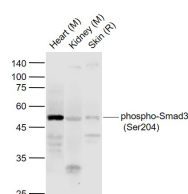
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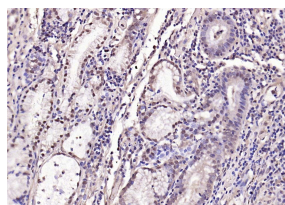
400-901-9800

— DATASHEET —**Host:** Rabbit**Isotype:** IgG**Clonality:** Polyclonal**GeneID:** 4088**SWISS:** P84022**Target:** Smad3 (Ser204)**Immunogen:** KLH conjugated Synthesised phosphopeptide derived from human Smad3 around the phosphorylation site of Ser204: AG(p-S)PN.**Purification:** affinity purified by Protein A**Concentration:** 1mg/ml**Storage:** 0.01M TBS (pH7.4) with 1% BSA, 0.02% Proclin300 and 50% Glycerol.

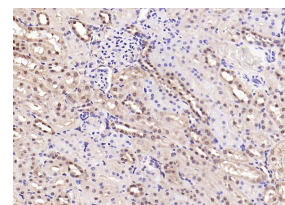
Shipped at 4°C. Store at -20°C for one year. Avoid repeated freeze/thaw cycles.

Background: Smad3 is a 50 kDa member of a family of proteins that act as key mediators of TGF beta superfamily signaling in cell proliferation, differentiation and development. The Smad family is divided into three subclasses: receptor regulated Smads, activin/TGF beta receptor regulated (Smad2 and 3) or BMP receptor regulated (Smad 1, 5, and 8); the common partner, (Smad4) that functions via its interaction to the various Smads; and the inhibitory Smads, (Smad6 and 7). Activated Smad3 oligomerizes with Smad4 upon TGF beta stimulation and translocates as a complex into the nucleus, allowing its binding to DNA and transcription factors. Phosphorylation of the two TGF beta dependent serines 423 and 425 in the C terminus of Smad3 is critical for Smad3 transcriptional activity and TGF beta signaling.**Applications:** WB (1:500-2000)**IHC-P** (1:100-500)**IHC-F** (1:100-500)**IF** (1:100-500)**Flow-Cyt** (1µg/Test)**ICC/IF** (1:100)**Reactivity:** Human, Mouse, Rat
(predicted: Rabbit, Pig, Sheep, Cow, Horse)**Predicted MW.:** 47 kDa**Subcellular Location:** Cytoplasm ,Nucleus**— VALIDATION IMAGES —**

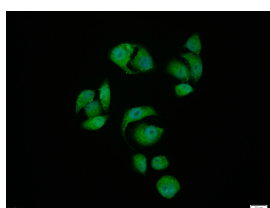
Sample: Lane 1: Heart (Mouse) Lysate at 40 ug
Lane 2: Kidney (Mouse) Lysate at 40 ug Lane 3: Skin (Rat) Lysate at 40 ug Primary: Anti-phospho-Smad3 (Ser204) (bs-5235R) at 1/1000 dilution Secondary: IRDye800CW Goat Anti-Rabbit IgG at 1/20000 dilution Predicted band size: 52 kD Observed band size: 50 kD



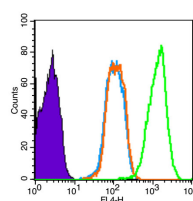
Paraformaldehyde-fixed, paraffin embedded (human gastric carcinoma); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15min; Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes; Blocking buffer (normal goat serum) at 37°C for 30min; Antibody incubation with (phospho-Smad3 (Ser204)) Polyclonal Antibody, Unconjugated (bs-5235R) at 1:200 overnight at 4°C, followed by operating according to SP Kit(Rabbit) (sp-0023) instructions and DAB staining.



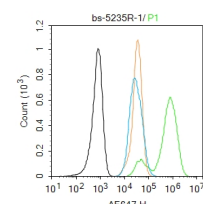
Paraformaldehyde-fixed, paraffin embedded (rat kidney); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15min; Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes; Blocking buffer (normal goat serum) at 37°C for 30min; Antibody incubation with (phospho-Smad3 (Ser204)) Polyclonal Antibody, Unconjugated (bs-5235R) at 1:200 overnight at 4°C, followed by operating according to SP Kit(Rabbit) (sp-0023) instructions and DAB staining.



HeLa cell; 4% Paraformaldehyde-fixed; Triton



Blank control (Black line): HUVEC (Black).



Blank control: A431. Primary Antibody (green)

Important Note: This product as supplied is intended for research use only, not for use in human, therapeutic or diagnostic applications.

X-100 at room temperature for 20 min; Blocking buffer (normal goat serum, C-0005) at 37°C for 20 min; Antibody incubation with (phospho-Smad3 (Ser204)) polyclonal Antibody, Unconjugated (bs-5235R) 1:100, 90 minutes at 37°C; followed by a conjugated Goat Anti-Rabbit IgG antibody at 37°C for 90 minutes, DAPI (blue, C02-04002) was used to stain the cell nuclei.

Primary Antibody (green line): Rabbit Anti-phospho-Smad3 (Ser204) antibody (bs-5235R) Dilution: 1µg /10⁶ cells; Isotype Control Antibody (orange line): Rabbit IgG . Secondary Antibody (white blue line): Goat anti-rabbit IgG-AF647 Dilution: 1µg /test. Protocol The cells were fixed with 4% PFA (10min at room temperature)and then permeabilized with 90% ice-cold methanol for 20 min at room temperature. The cells were then incubated in 5%BSA to block non-specific protein-protein interactions for 30 min at room temperature .Cells stained with Primary Antibody for 30 min at room temperature. The secondary antibody used for 40 min at room temperature. Acquisition of 20,000 events was performed.

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— SELECTED CITATIONS —

- **[IF=4.175]** Huajun Wang. et al. LncRNA NEAT1 promotes proliferation, migration, invasion and epithelial-mesenchymal transition process in TGF-β2-stimulated lens epithelial cells through regulating the miR-486-5p/SMAD4 axis. Cancer Cell Int. 2020 Dec;20(1):1-12 WB ;Human. 33292220
- **[IF=3.1]** Zhang, Hongjun, et al. "Magnolol Attenuates Concanavalin A - induced Hepatic Fibrosis, Inhibits CD4+ T Helper 17 (Th17) Cell Differentiation and Suppresses Hepatic Stellate Cell Activation: Blockade of Smad3/Smad4 Signalling." Basic & Clinical Pharmacology & Toxicology (2016). WB ;="Mouse". 28032440
- **[IF=3.3]** Kaiqiang Meng. et al.The mutualistic relationship between M2c macrophages of TGFβ1 induction and gastric cancer cells: the correlation between protective mechanisms in the tumor microenvironment and polarization of subtypes of cells.JOURNAL OF CANCER.2025 Feb 3;16(5):1598-1617. Western Blot ;human. 39991579
- **[IF=3.3]** Kaiqiang Meng. et al.The mutualistic relationship between M2c macrophages of TGFβ1 induction and gastric cancer cells: the correlation between protective mechanisms in the tumor microenvironment and polarization of subtypes of cells.JOURNAL OF CANCER.2025 Feb 3;16(5):1598-1617. Western blot ;Human. 10.7150/jca.97784