bs-2225R **[Primary Antibody]**

phospho-Smad3 (Ser423) Rabbit pAb

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DATASHEET

Host: Rabbit **Isotype:** IgG

Clonality: Polyclonal

GeneID: 4088 **SWISS:** P84022

Target: Smad3 (Ser423)

Immunogen: KLH conjugated Synthesised phosphopeptide derived from human Smad3 around the phosphorylation site of Ser423: CS(p-S)VS.

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.

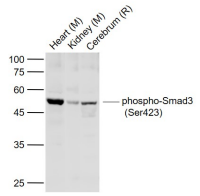
Background: Smad3 is a 50 kDa member of a family of proteins that act as key mediators of TGF beta superfamily signaling in cell proliferation, differentiation and development. The Smad family is divided into three subclasses: receptor regulated Smads, activin/TGF beta receptor regulated (Smad2 and 3) 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)
Flow-Cyt (3μg/Test)
ICC/IF (1:100)

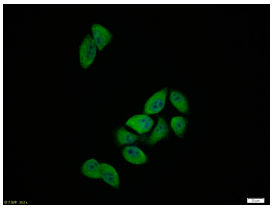
Reactivity: Human, Mouse, Rat (predicted: Pig)

Subcellular Location: Cytoplasm ,Nucleus

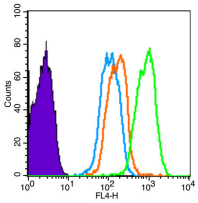
VALIDATION IMAGES



Sample: Lane 1: Heart (Mouse) Lysate at 40 ug Lane 2: Kidney (Mouse) Lysate at 40 ug Lane 3: Cerebrum (Rat) Lysate at 40 ug Primary: Anti-phospho-Smad3 (Ser423) (bs-2225R) at 1/1000 dilution Secondary: IRDye800CW Goat Anti-Rabbit IgG at 1/20000 dilution Predicted band size: 52 kD Observed band size: 52 kD



Hela cell; 4% Paraformaldehyde-fixed; Triton 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 (Ser423)) polyclonal Antibody, Unconjugated (bs-2225R) 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.



Blank control (Black line): HUVEC (Black). Primary Antibody (green line): Rabbit Anti-phospho-Smad3 (Ser423) antibody (bs-2225R) Dilution: 3μg /10^6 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.

SELECTED CITATIONS

- **[IF=5.714]** Han B et al. Deltamethrin induces liver fibrosis in quails via activation of the TGF-β1/Smad signaling pathway. Environ Pollut. 2019 Dec 23;259:113870. WB ;quail. 31918140
- **[IF=5.589]** Lv Y et al. Imidacloprid-induced liver fibrosis in quails via activation of the TGF-β1/Smad pathway. Sci Total Environ. 2019 Dec 6;705:135915. WB ;Quail. 31835194
- **[IF=4.784]** Zheng Wu. et al. FOXD3 suppresses epithelial–mesenchymal transition through direct transcriptional promotion of SMAD7 in esophageal squamous cell carcinoma. 2021 Sep 22 WB ;human. 34551139