bs-5617R **[Primary Antibody]**

phospho-Smad3 (Ser208) Rabbit pAb

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DATASHEET

Host: Rabbit **Isotype:** IgG

Clonality: Polyclonal

GeneID: 4088 **SWISS:** P84022

Target: Smad3 (Ser208)

Immunogen: KLH conjugated Synthesised phosphopeptide derived from human Smad3 around the phosphorylation site of Ser208: NL(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.

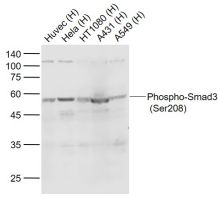
Applications: **WB** (1:500-2000)
IHC-P (1:100-500)
IHC-F (1:100-500)
IF (1:100-500)
Flow-Cyt (1μg/Test)

Reactivity: Human, Rat (predicted: Mouse)

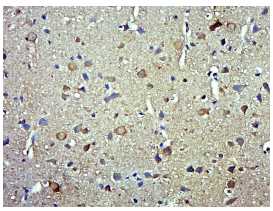
Predicted MW.: 47 kDa

Subcellular Location: Cytoplasm ,Nucleus

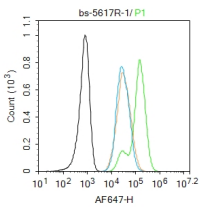
VALIDATION IMAGES



Sample: Lane 1: Huvec (Human) Cell Lysate at 30 ug Lane 2: Hela (Human) Cell Lysate at 30 ug Lane 3: HT1080 (Human) Cell Lysate at 30 ug Lane 4: A431 (Human) Cell Lysate at 30 ug Lane 5: A549 (Human) Cell Lysate at 30 ug Primary: Anti-Phospho-Smad3 (Ser208) (bs-5617R) at 1/1000 dilution Secondary: IRDye800CW Goat Anti-Rabbit IgG at 1/20000 dilution Predicted band size: 52 kD Observed band size: 54 kD



Paraformaldehyde-fixed, paraffin embedded (Rat brain); 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 (Ser208)) Polyclonal Antibody, Unconjugated (bs-5617R) at 1:500 overnight at 4°C, followed by a conjugated secondary (sp-0023) for 20 minutes and DAB staining.



Blank control:A431. Primary Antibody (green line): Rabbit Anti-Phospho-Smad3 (Ser208) antibody (bs-5617R) Dilution: 1μg /10^6 cells; Isotype Control Antibody (orange line): Rabbit IgG . Secondary Antibody : 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 -20°C.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=5.6]** Shuang Liu. et al. Periodontal ligament-associated protein-1 knockout mice regulate the differentiation of

osteoclasts and osteoblasts through TGF-β1/Smad signaling pathway. J CELL PHYSIOL. 2023 Jun;: WB ;Mouse. 37357387

- **[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