bs-3425R **[Primary Antibody]**

phospho-Smad3 (Ser423 + Ser425) Rabbit pAb

www.bioss.com.cn
sales@bioss.com.cn
techsupport@bioss.com.cn
400-901-9800

DATASHEET

Host: Rabbit **Isotype:** IgG

Clonality: Polyclonal

GeneID: 4088 **SWISS:** P84022

Target: Smad3 (Ser423 + Ser425)

Immunogen: KLH conjugated Synthesised phosphopeptide derived from human Smad3 around the phosphorylation site of Ser423/425: CS(p-S)V(p-S).

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.

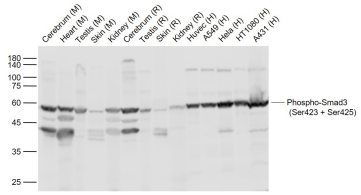
Applications: **WB** (1:500-2000)
IHC-P (1:100-500)
IHC-F (1:100-500)
IF (1:100-500)
Flow-Cyt (1μg/Test)

Reactivity: Human, Mouse, Rat (predicted: Pig, Cow, Chicken, Dog, Horse)

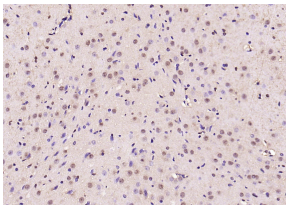
Predicted MW.: 47 kDa

Subcellular Location: Cytoplasm ,Nucleus

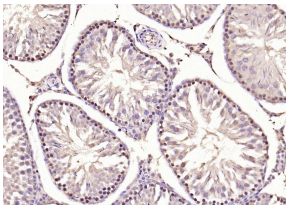
VALIDATION IMAGES



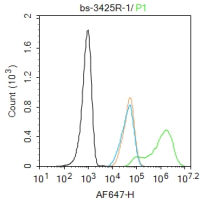
Sample: Lane 1: Cerebrum (Mouse) Lysate at 40 ug Lane 2: Heart (Mouse) Lysate at 40 ug Lane 3: Testis (Mouse) Lysate at 40 ug Lane 4: Skin (Mouse) Lysate at 40 ug Lane 5: Kidney (Mouse) Lysate at 40 ug Lane 6: Cerebrum (Rat) Lysate at 40 ug Lane 7: Testis (Rat) Lysate at 40 ug Lane 8: Skin (Rat) Lysate at 40 ug Lane 9: Kidney (Rat) Lysate at 40 ug Lane 10: Huvec (Human) Cell Lysate at 30 ug Lane 11: A549 (Human) Cell Lysate at 30 ug Lane 12: Hela (Human) Cell Lysate at 30 ug Lane 13: HT1080 (Human) Cell Lysate at 30 ug Lane 14: A431 (Human) Cell Lysate at 30 ug Primary: Anti-Phospho-Smad3 (Ser423 + Ser425) (bs-3425R) at 1/1000 dilution Secondary: IRDye800CW Goat Anti-Rabbit IgG at 1/20000 dilution Predicted band size: 52 kD Observed band size: 54 kD



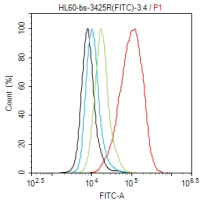
Paraformaldehyde-fixed, paraffin embedded (rat brain); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15min; Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes; Blocking buffer (normal goat serum) at 37°C for 30min; Antibody incubation with (Phospho-Smad3 (Ser423 + Ser425)) Polyclonal Antibody, Unconjugated (bs-3425R) at 1:200 overnight at 4°C, followed by operating according to SP Kit(Rabbit) (sp-0023) instructionsand DAB staining.



Paraformaldehyde-fixed, paraffin embedded (rat testis); 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 (Ser423 + Ser425)) Polyclonal Antibody, Unconjugated (bs-3425R) at 1:200 overnight at 4°C, followed by operating according to SP Kit(Rabbit) (sp-0023) instructionsand DAB staining.



Blank control: Hela. Primary Antibody (green line): Rabbit Anti-Phospho-Smad3 (Ser423 + Ser425) antibody (bs-3425R) 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.



Blank control (black line): HL60(black) (The cells were fixed with 2% paraformaldehyde (10 min) , then permeabilized with PBST for 30 min on room temperature) Primary Antibody (Red line): Rabbit Anti-Phopho-Smad3(Ser423+Ser425) antibody (bs-3152R) ; Dilution: 1μg /10^6 cells; Isotype Control Antibody (green line): Rabbit IgG . Secondary Antibody (red line): Goat anti-rabbit IgG-FITC;Dilution: 1μg /test.

SELECTED CITATIONS

- **[IF=17.694]** Kim, Duk Ki. et al. PD-L1-directed PlGF/VEGF blockade synergizes with chemotherapy by targeting CD141+ cancer-associated fibroblasts in pancreatic cancer. NAT COMMUN. 2022 Oct;13(1):1-19 FCM ;Mouse. 36272973
- **[IF=14]** Ke Xu. et al. Polystyrene microplastics and di-2-ethylhexyl phthalate co-exposure: Implications for female reproductive health. Environmental Science and Ecotechnology. 2024 Nov;22:100471 IHC ;Rat. 39220680
- **[IF=9.7]** Xing Li. et al. Inducible nitric oxide synthase (iNOS)-activated Cxcr2 signaling in myeloid cells promotes TGFβ-dependent squamous cell carcinoma lung metastasis. CANCER LETT. 2023 Aug;570:216330 IHC ;Human. 37524225
- **[IF=8.5]** Genghua Chen. et al. Bulk and single-cell alternative splicing analyses reveal roles of TRA2B in myogenic differentiation. CELL PROLIFERAT. 2023 Sep;:e13545 WB ;Chicken. 37705195
- **[IF=8.3]** Cai Bolin. et al. MYH1G-AS is a chromatin-associated lncRNA that regulates skeletal muscle development in chicken. CELL MOL BIOL LETT. 2024 Dec;29(1):1-25 WB ;Chicken. 38177995