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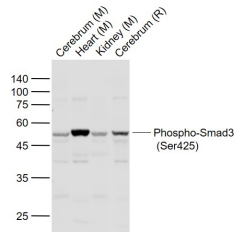
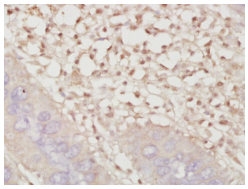
Phospho-Smad3 (Ser425) Rabbit pAb

Catalog Number:	bs-5616R
Target Protein:	Phospho-Smad3 (Ser425)
Concentration:	1mg/ml
Form:	Liquid
Host:	Rabbit
Clonality:	Polyclonal
Isotype:	IgG
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:Rabbit, Pig, Cow, Chicken, Horse)
Predicted MW:	47 kDa
Entrez Gene:	4088
Swiss Prot:	P84022
Source:	KLH conjugated Synthesised phosphopeptide derived from human Smad3 around the phosphorylation site of Ser425: SV(p-S)-NH2.
Purification:	affinity purified by Protein A
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.

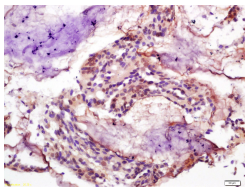
VALIDATION IMAGES



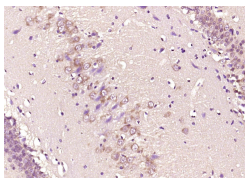
Sample: Lane 1: Cerebrum (Mouse) Lysate at 40 ug Lane 2: Heart (Mouse) Lysate at 40 ug Lane 3: Kidney (Mouse) Lysate at 40 ug Lane 4: Cerebrum (Rat) Lysate at 40 ug Primary: Anti- Phospho-Smad3 (Ser425) (bs-5616R) at 1/1000 dilution Secondary: IRDye800CW Goat Anti-Rabbit IgG at 1/20000 dilution Predicted band size: 52 kD Observed band size: 50 kD



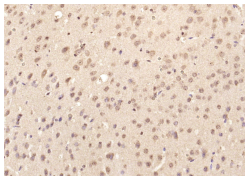
Paraformaldehyde-fixed, paraffin embedded (Human colon cancer); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15min; Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes; Blocking buffer (normal goat serum) at 37°C for 30min; Antibody incubation with (Phospho-Smad3 (Ser425)) Polyclonal Antibody, Unconjugated (bs-5616R) at 1:400 overnight at 4°C, followed by a conjugated secondary antibody (sp-0023) for 20 minutes and DAB staining.



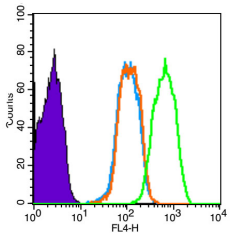
Tissue/cell: human colon carcinoma; 4% Paraformaldehyde-fixed and paraffin-embedded; Antigen retrieval: citrate buffer (0.01M, pH 6.0), Boiling bathing for 15min; Block endogenous peroxidase by 3% Hydrogen peroxide for 30min; Blocking buffer (normal goat serum,C-0005) at 37°C for 20 min; Incubation: Anti-Phospho-Smad3(Ser425) Polyclonal Antibody, Unconjugated(bs-5616R) 1:200, overnight at 4°C, followed by conjugation to the secondary antibody(SP-0023) and DAB(C-0010) staining



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 (Ser425)) Polyclonal Antibody, Unconjugated (bs-5616R) at 1:200 overnight at 4°C, followed by operating according to SP Kit(Rabbit) (sp-0023) instructionsand DAB staining.



Paraformaldehyde-fixed, paraffin embedded (mouse 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 (Ser425)) Polyclonal Antibody, Unconjugated (bs-5616R) at 1:200 overnight at 4°C, followed by operating according to SP Kit(Rabbit) (sp-0023) instructionsand DAB staining.



Blank control (Black line): HUVEC (Black). Primary Antibody (green line): Rabbit Anti-Phospho-Smad3 (Ser425) antibody (bs-5616R) Dilution: 1µg /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.

PRODUCT SPECIFIC PUBLICATIONS

[IF=4.545] Han X et al. The intervention effect of nicotine on cervical fibroblast-myofibroblast differentiation in lipopolysaccharide-induced preterm birth model through activating the TGF-β1/Smad3 pathwayBiomed Pharmacother.2020 Dec 24;134:111135. WB ; Mouse . 33352448

[IF=4.271] Biao Li. et al. 20-Hydroxytetraenoic acid induces hepatic fibrosis via the TGF-β1/Smad3 signaling pathway. TOXICOL LETT. 2023 Jan;373:1 WB ; Human, Mouse . 36368619

[IF=2.728] Xijuan Liu et al. Chondrocyte suppression is mediated by miR‐129‐5p via GDF11/SMAD3 signaling in developmental

dysplasia of the hip. J Orthop Res. 2020 Dec;38(12):2559-2572. WB ; Rabbit . 32396235