

Product Datasheet

Phospho-Smad3 (Ser204) Rabbit Polyclonal Antibody (orb4684)

Catalog Number	orb4684
Category	Antibodies
Description	Phospho-Smad3 (Ser204) Rabbit Polyclonal Antibody
Target	SMAD3
Clonality	Polyclonal
Species/Host	Rabbit
Isotype	IgG
Conjugation	Unconjugated
Reactivity	Human, Mouse, Rat
Predicted Reactivity	Bovine, Equine, Porcine, Rabbit, Sheep
Form/Appearance	Liquid
Concentration	1mg/ml
Buffer/Preservatives	0.01M TBS (pH7.4) with 1% rAlbumin, 0.02% Proclin300 and 50% Glycerol.
Purification	Affinity purified by Protein A
Immunogen	KLH conjugated Synthesised phosphopeptide derived from human Smad3 around the phosphorylation site of Ser204 AG(p-S)PN
UniProt ID	P84022

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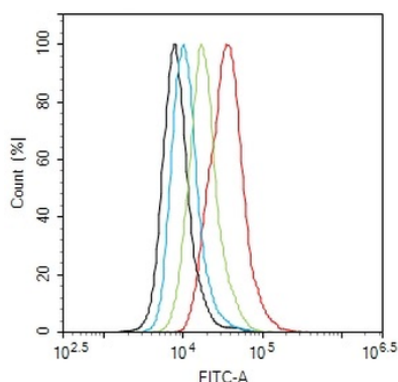
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RRID	AB_10925437
MW	50 kDa
Tested applications	FC, ICC, IF, IHC-Fr, IHC-P, WB
Dilution range	WB=1:500-2000, IHC-P=1:100-500, IHC-F=1:100-500, ICC/IF=1:100-500, IF=1:100-500, Flow-Cyt=1µg/Test
Antibody Type	Primary Antibody
Storage	Maintain refrigerated at 2-8°C for up to 2 weeks. For long term storage store at -20°C in small aliquots to prevent freeze-thaw cycles.
Note	For research use only
Expiration Date	12 months from date of receipt.



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 (green line): Rabbit Anti-Phospho-Smad3 (Ser204) antibody (orb4684), dilution: 1 µg/10⁶ cells, Isotype Control Antibody (orange line): Rabbit IgG. Secondary Antibody (white blue line): Goat anti-rabbit IgG-FITC, dilution: 1 µg/Test.

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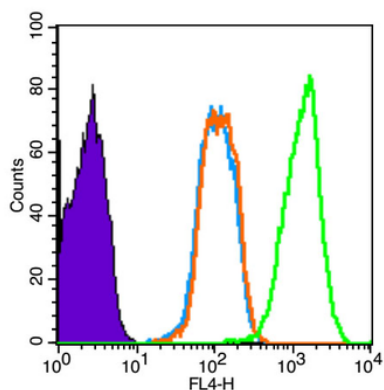
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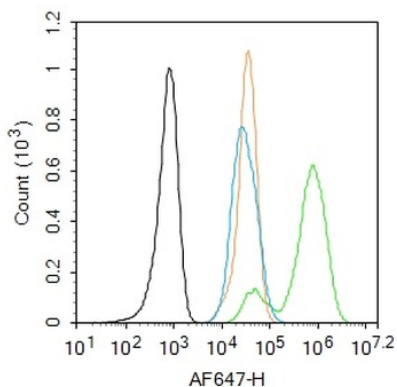
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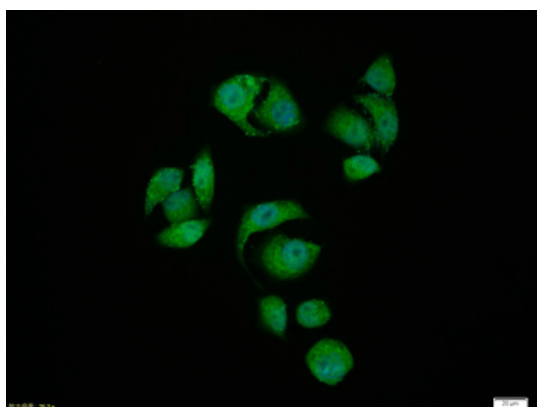
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Blank control (Black line): HUVEC (Black). Primary Antibody (green line): Rabbit Anti-phospho-Smad3 (Ser204) antibody (orb4684), dilution: 1 $\mu\text{g}/10^6$ cells, Isotype Control Antibody (orange line): Rabbit IgG. Secondary Antibody (white blue line): Goat anti-rabbit IgG-AF647, dilution: 1 $\mu\text{g}/\text{Test}$. Protocol, The cells were fixed with 4% PFA (10 min 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 20000 events was performed.



Blank control: A431. Primary Antibody (green line): Rabbit Anti-phospho-Smad3 (Ser204) antibody (orb4684), dilution: 1 $\mu\text{g}/10^6$ cells, Isotype Control Antibody (orange line): Rabbit IgG. Secondary Antibody: Goat anti-rabbit IgG-AF647, dilution: 1 $\mu\text{g}/\text{Test}$. Protocol, The cells were fixed with 4% PFA (10 min 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 20000 events was performed.



Hela cell, 4% Paraformaldehyde-fixed, Triton X-100 at room temperature for 20 min, Blocking buffer (normal goat serum) at 37°C for 20 min, Antibody incubation with (phospho-Smad3 (Ser204)) polyclonal Antibody, Unconjugated (orb4684) 1:100, 90 minutes at 37°C , followed by a conjugated Goat Anti-Rabbit IgG antibody at 37°C for 90 minutes, DAPI (blue) was used to stain the cell nuclei.

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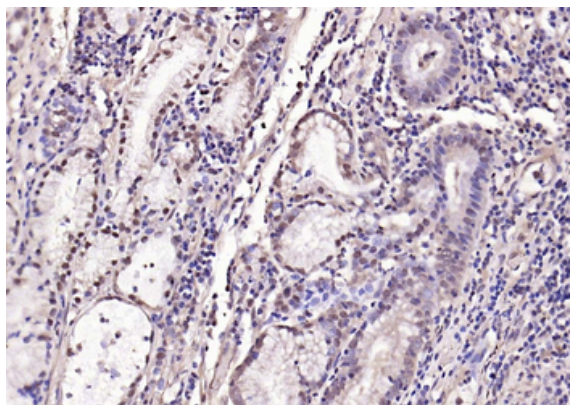
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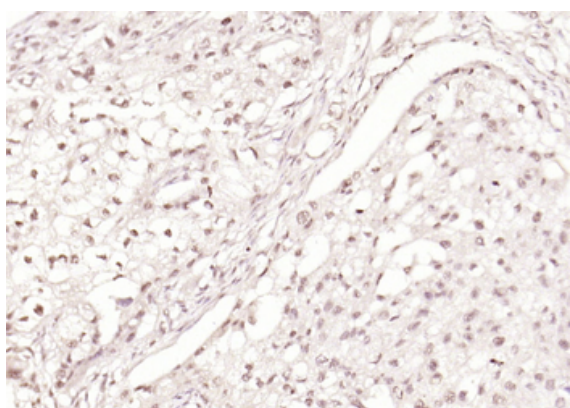
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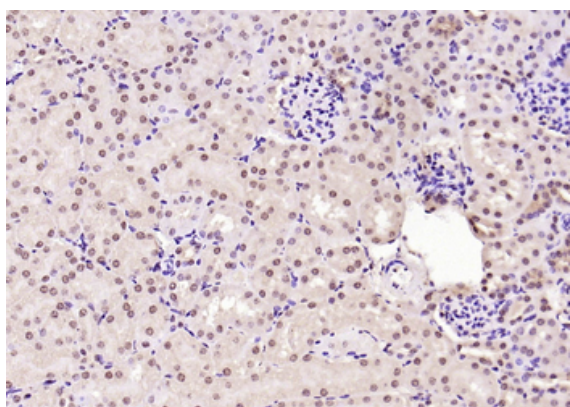
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Paraformaldehyde-fixed, paraffin embedded (human gastric carcinoma), Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15 min, Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes, Blocking buffer (normal goat serum) at 37°C for 30 min, Antibody incubation with (phospho-Smad3 (Ser204)) Polyclonal Antibody, Unconjugated (orb4684) at 1:200 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.



Paraformaldehyde-fixed, paraffin embedded (human skin cancer), Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15 min, Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes, Blocking buffer (normal goat serum) at 37°C for 30 min, Antibody incubation with (phospho-Smad3 (Ser204)) Polyclonal Antibody, Unconjugated (orb4684) at 1:200 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.



Paraformaldehyde-fixed, paraffin embedded (mouse kidney), Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15 min, Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes, Blocking buffer (normal goat serum) at 37°C for 30 min, Antibody incubation with (phospho-Smad3 (Ser204)) Polyclonal Antibody, Unconjugated (orb4684) at 1:200 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.

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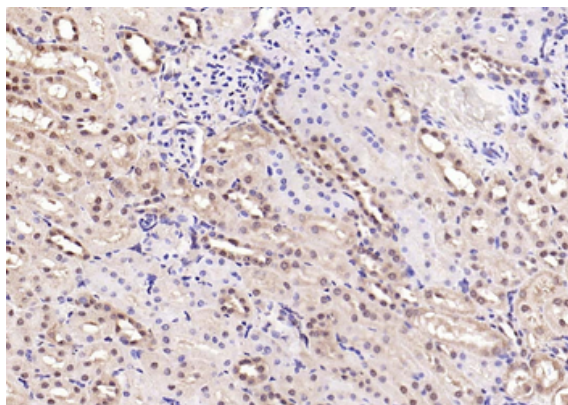
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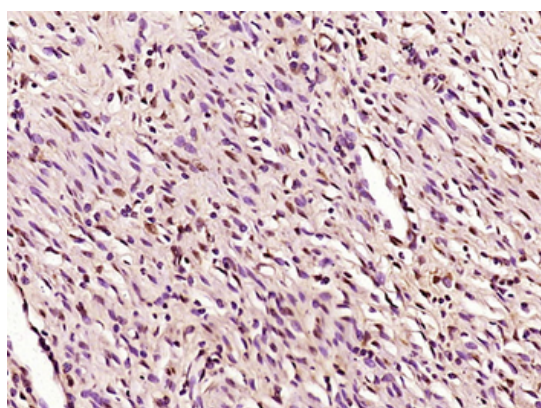
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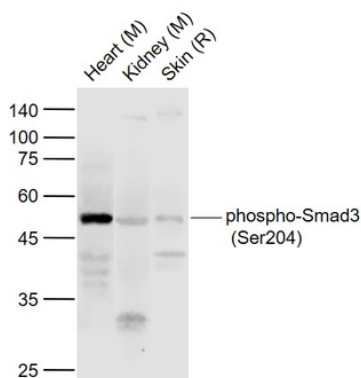
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Paraformaldehyde-fixed, paraffin embedded (rat kidney), Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15 min, Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes, Blocking buffer (normal goat serum) at 37°C for 30 min, Antibody incubation with (phospho-Smad3 (Ser204)) Polyclonal Antibody, Unconjugated (orb4684) at 1:200 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.



Paraformaldehyde-fixed, paraffin embedded (Rat uterus), Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15 min, Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes, Blocking buffer (normal goat serum) at 37°C for 30 min, Antibody incubation with (phospho-Smad3 (Ser204)) Polyclonal Antibody, Unconjugated (orb4684) at 1:400 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.



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) (orb4684) at 1/1000 dilution, Secondary: IRDye800CW Goat Anti-Rabbit IgG at 1/20000 dilution, Predicted band size: 52 kD, Observed band size: 50 kD.

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