

Product Datasheet

Phospho-Smad3 (Ser423 + Ser425) Rabbit Polyclonal Antibody (orb6983)

Catalog Number	orb6983
Category	Antibodies
Description	Rabbit Anti-Phospho-Smad3 (Ser423 + Ser425) antibody
Target	SMAD3
Clonality	Polyclonal
Species/Host	Rabbit
Isotype	IgG
Conjugation	Unconjugated
Reactivity	Human, Mouse, Rat
Predicted Reactivity	Bovine, Canine, Equine, Gallus, Porcine
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 Ser423/425 CS(p-S)V(p-S)
UniProt ID	P84022

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7 Signet Court, Swann Road
Cambridge
CB5 8LA
United Kingdom

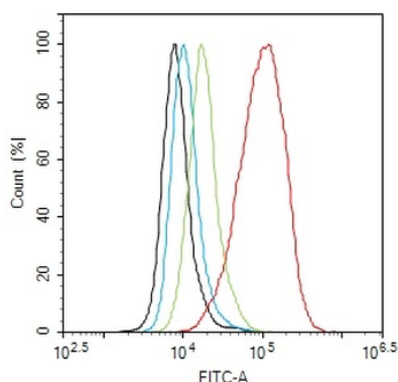
Email: info@biorbyt.com, support@biorbyt.com
Phone: [+44 \(0\)1223 859353](tel:+44(0)1223859353) | Fax: [+1 \(415\) 651-8558](tel:+1(415)651-8558)

Biorbyt LLC

68 TW Alexander Drive
Research Triangle Park
Durham
NC 27713
United States

Email: info@biorbyt.com, support@biorbyt.com
Phone: [+1 \(415\) 906-5211](tel:+1(415)906-5211) | Fax: [+1 \(415\) 651-8558](tel:+1(415)651-8558)

RRID	AB_10935296
MW	47 kDa
Tested applications	FC, IF, IHC-Fr, IHC-P, WB
Dilution range	WB=1:500-2000, IHC-P=1:100-500, IHC-F=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 (Red line): Rabbit Anti-Phospho-Smad3 (Ser423+Ser425) antibody, dilution: 1 µg/10⁶ cells, Isotype Control Antibody (green line): Rabbit IgG. Secondary Antibody (red line): Goat anti-rabbit IgG-FITC, dilution: 1 µg/Test.

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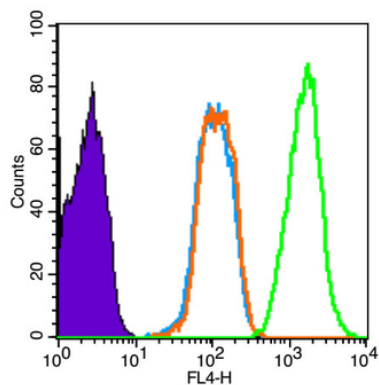
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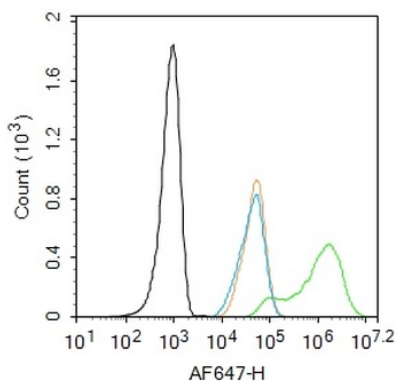
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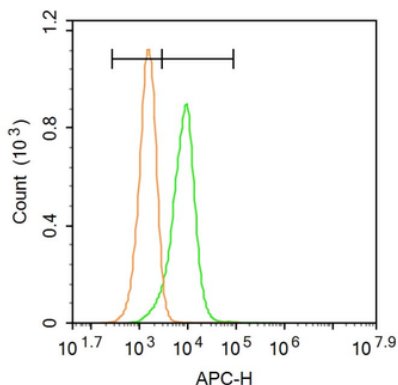
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Blank control (Black line): HUVEC (Black). Primary Antibody (green line): Rabbit Anti-Phospho-Smad3 (Ser423 + Ser425) antibody (orb6983), dilution: 1 $\mu\text{g}/10^6$ cells, Isotype Control Antibody (orange line): Rabbit IgG. Secondary Antibody (white 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: HeLa. Primary Antibody (green line): Rabbit Anti-Phospho-Smad3 (Ser423 + Ser425) antibody (orb6983), 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.



Blank control: HUVEC (Black). Primary Antibody (green line): Rabbit Anti-Phospho-Jak1 antibody, 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 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.

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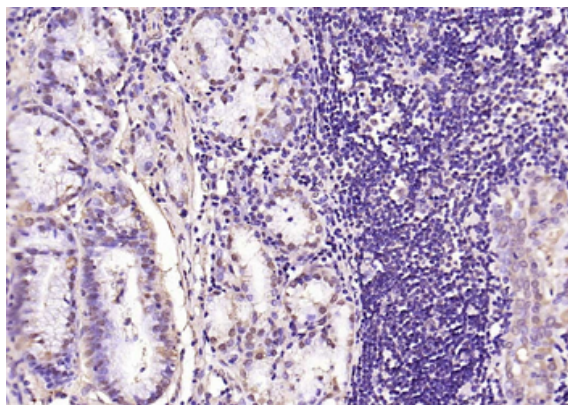
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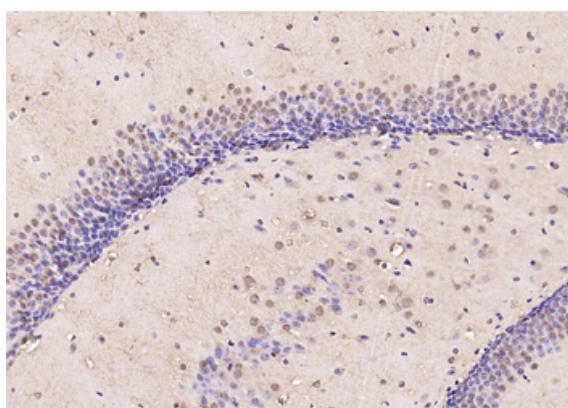
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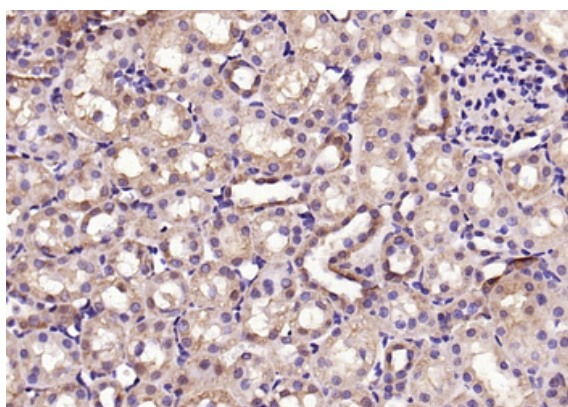
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Paraformaldehyde-fixed, paraffin embedded (human gastric carcinoma), Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15 min, Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes, Blocking buffer (normal goat serum) at 37°C for 30 min, Antibody incubation with (Phospho-Smad3 (Ser423 + Ser425)) Polyclonal Antibody, Unconjugated (orb6983) at 1:200 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.



Paraformaldehyde-fixed, paraffin embedded (mouse brain), 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 (Ser423 + Ser425)) Polyclonal Antibody, Unconjugated (orb6983) 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 (Ser423 + Ser425)) Polyclonal Antibody, Unconjugated (orb6983) at 1:200 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.

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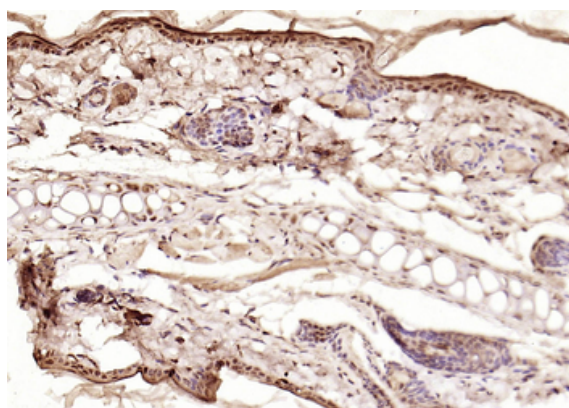
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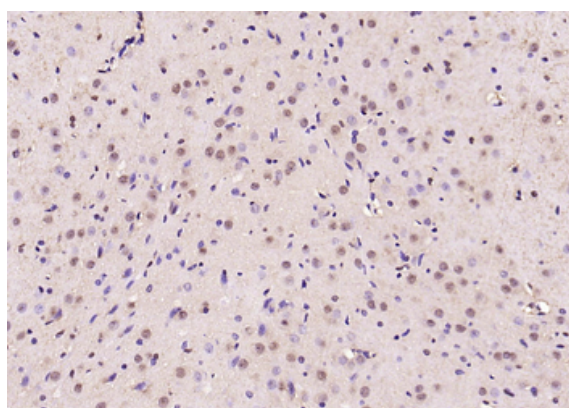
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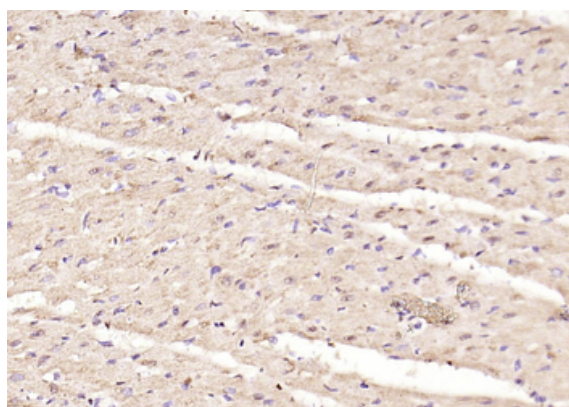
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Paraformaldehyde-fixed, paraffin embedded (mouse skin), Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15 min, Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes, Blocking buffer (normal goat serum) at 37°C for 30 min, Antibody incubation with (Phospho-Smad3 (Ser423 + Ser425)) Polyclonal Antibody, Unconjugated (orb6983) at 1:200 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.



Paraformaldehyde-fixed, paraffin embedded (rat brain), 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 (Ser423 + Ser425)) Polyclonal Antibody, Unconjugated (orb6983) at 1:200 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.



Paraformaldehyde-fixed, paraffin embedded (rat heart), 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 (Ser423 + Ser425)) Polyclonal Antibody, Unconjugated (orb6983) at 1:200 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.

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Cambridge
CB5 8LA
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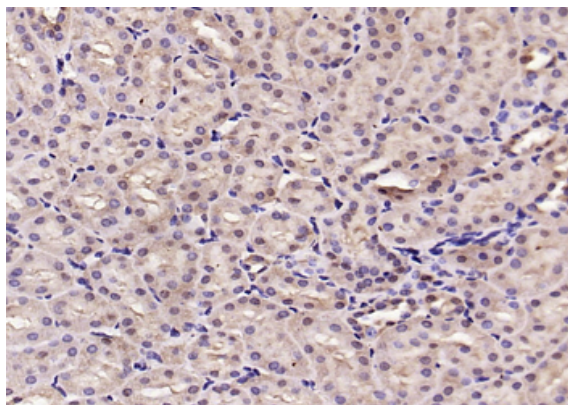
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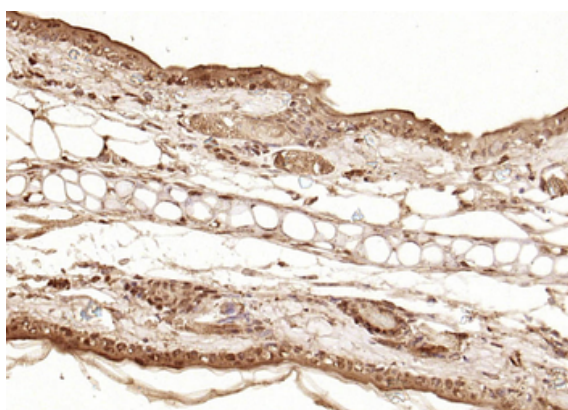
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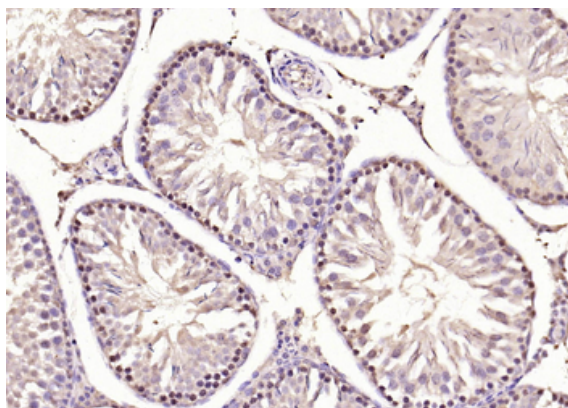
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Paraformaldehyde-fixed, paraffin embedded (rat kidney), Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15 min, Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes, Blocking buffer (normal goat serum) at 37°C for 30 min, Antibody incubation with (Phospho-Smad3 (Ser423 + Ser425)) Polyclonal Antibody, Unconjugated (orb6983) at 1:200 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.



Paraformaldehyde-fixed, paraffin embedded (rat skin), 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 (Ser423 + Ser425)) Polyclonal Antibody, Unconjugated (orb6983) at 1:200 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.



Paraformaldehyde-fixed, paraffin embedded (rat testis), 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 (Ser423 + Ser425)) Polyclonal Antibody, Unconjugated (orb6983) at 1:200 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.

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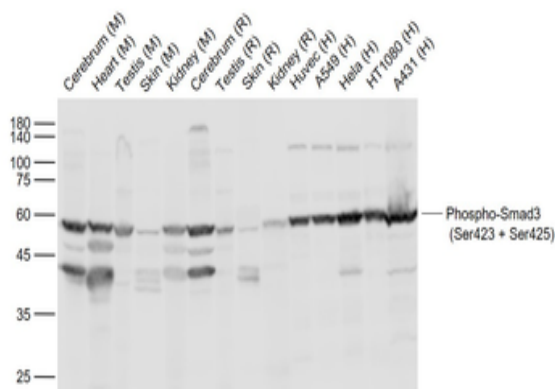
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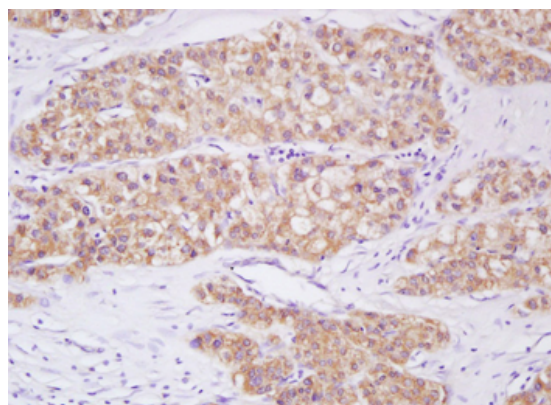
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Sample: Lane 1: Cerebrum (Mouse) Lysate at 40 ug, Lane 2: Heart (Mouse) Lysate at 40 ug, Lane 3: Testis (Mouse) Lysate at 40 ug, Lane 4: Skin (Mouse) Lysate at 40 ug, Lane 5: Kidney (Mouse) Lysate at 40 ug, Lane 6: Cerebrum (Rat) Lysate at 40 ug, Lane 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) (orb6983) at 1/1000 dilution, Secondary: IRDye800CW Goat Anti-Rabbit IgG at 1/20000 dilution, Predicted band size: 52 kD, Observed band size: 54 kD.



Tissue/Cell: human liver carcinoma, 4% Paraformaldehyde-fixed and paraffin-embedded, Antigen retrieval: citrate buffer (0.01M, pH6.0), Boiling bathing for 15 min, Block endogenous peroxidase by 3% Hydrogen peroxide for 30 min, Blocking buffer (normal goat serum) at 37°C for 20 min, Incubation: Anti-Phospho-Smad3 (Ser423/425) Polyclonal Antibody, Unconjugated (orb6983) 1:200, overnight at 4°C, followed by conjugation to the secondary antibody and DAB staining.

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