

## Product Datasheet

### HMGB1 Rabbit Polyclonal Antibody (orb500823)

<b>Catalog Number</b>	orb500823
<b>Category</b>	Antibodies
<b>Description</b>	HMGB1 Rabbit Polyclonal Antibody
<b>Target</b>	HMGB1
<b>Clonality</b>	Polyclonal
<b>Species/Host</b>	Rabbit
<b>Isotype</b>	IgG
<b>Conjugation</b>	Unconjugated
<b>Reactivity</b>	Human, Mouse, Rat
<b>Predicted Reactivity</b>	Bovine, Canine, Equine, Porcine
<b>Form/Appearance</b>	Liquid
<b>Concentration</b>	1mg/ml
<b>Buffer/Preservatives</b>	0.01M TBS (pH7.4) with 1% rAlbumin, 0.02% Proclin300 and 50% Glycerol.
<b>Purification</b>	Affinity purified by Protein A
<b>Immunogen</b>	KLH conjugated synthetic peptide derived from mouse HMGB1 (61-150/215aa)
<b>MW</b>	25 kDa
<b>Tested applications</b>	FC, ICC, IF, IHC-Fr, IHC-P

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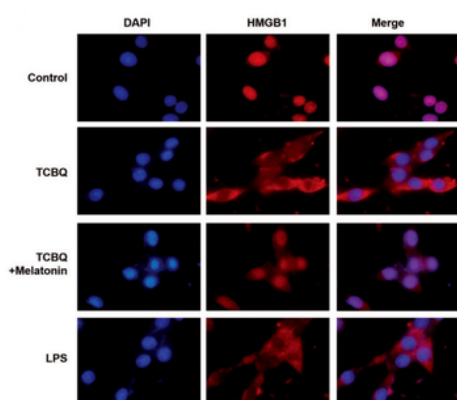
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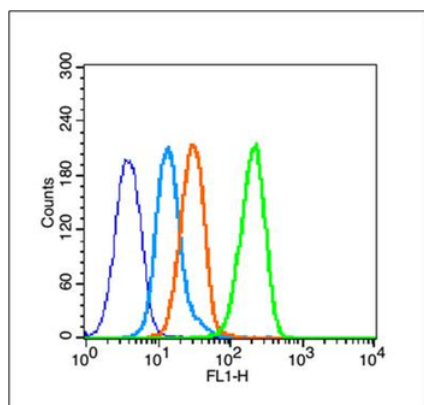
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<b>Dilution range</b>	IHC-P=1:100-500, IHC-F=1:100-500, ICC/IF=1:100-500, IF=1:100-500, Flow-Cyt=1µg/Test
<b>Antibody Type</b>	Primary Antibody
<b>Storage</b>	Maintain refrigerated at 2-8°C for up to 2 weeks. For long term storage store at -20°C in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



4% Paraformaldehyde fixed PC12 cells stained with Rabbit Anti-HMGB1 Polyclonal Antibody (orb500823) at 1:300 for 3 hours at 4°C, followed by Rhodamine-conjugated secondary antibody for an additional hour.



Blank control (blue line): MCF7 (fixed with 80% ethanol (Overnight at 4°C) and then permeabilized with 90% ice-cold methanol for 30 min on ice). Primary Antibody (green line): Rabbit Anti-HMGB1 antibody (orb500823), dilution: 1 µg/10<sup>6</sup> 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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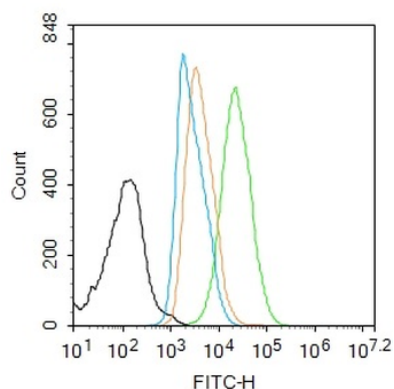
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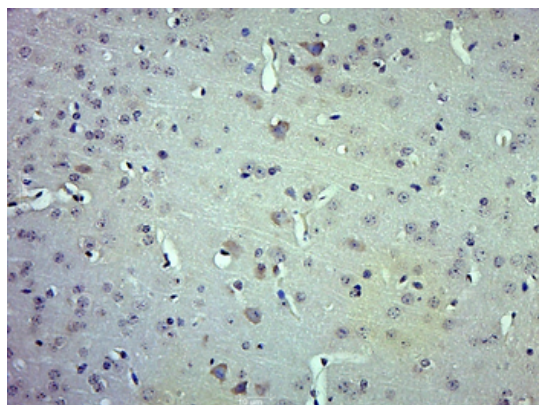
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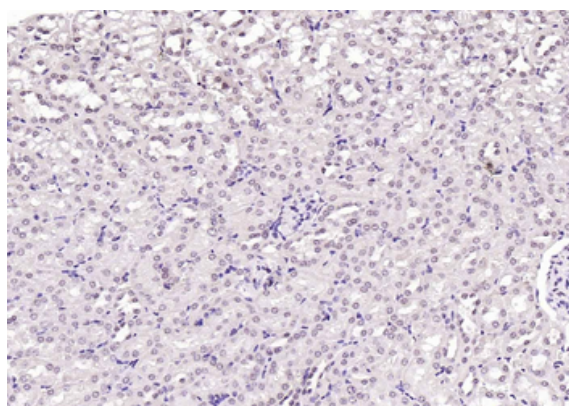
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Blank control: HL-60. Primary Antibody (green line): Rabbit Anti-HMGB1 antibody (orb500823), dilution: 1  $\mu\text{g}/10^6$  cells, Isotype Control Antibody (orange line): Rabbit IgG. Secondary Antibody: Goat anti-rabbit IgG-AF488, dilution: 1  $\mu\text{g}/\text{Test}$ . 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.



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 (HMGB1) Polyclonal Antibody, Unconjugated (orb500823) at 1:500 overnight at 4°C, followed by a conjugated secondary for 20 minutes 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, Incubation with (HMGB1) Polyclonal Antibody, Unconjugated (orb500823) at 1:200 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.

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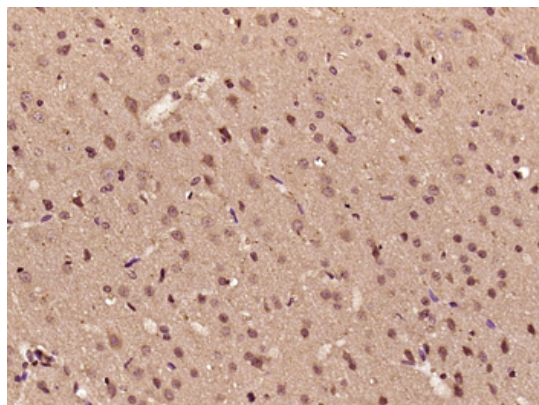
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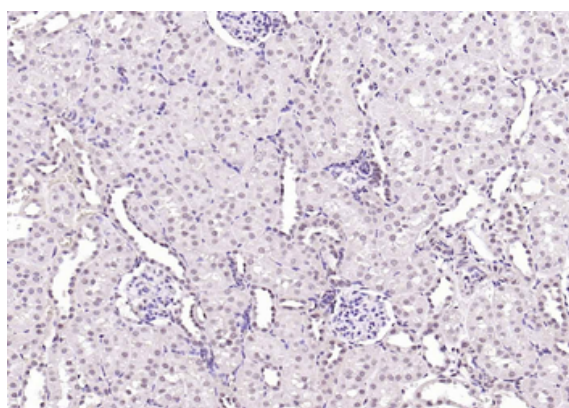
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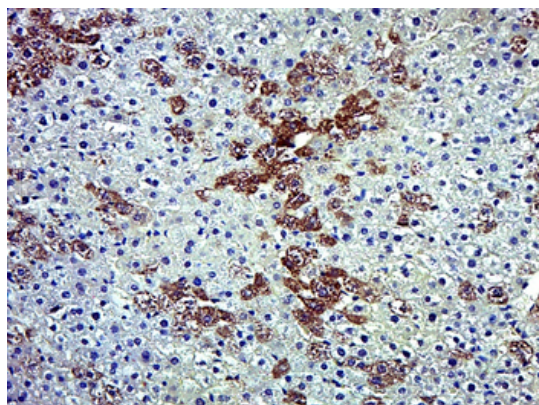
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Paraformaldehyde-fixed, paraffin embedded (Rat brain), Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15 min, Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes, Blocking buffer (normal goat serum) at 37°C for 30 min, Antibody incubation with (HMGB1) Polyclonal Antibody, Unconjugated (orb500823) at 1:500 overnight at 4°C, followed by a conjugated secondary for 20 minutes and DAB staining.



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, Incubation with (HMGB1) Polyclonal Antibody, Unconjugated (orb500823) at 1:200 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.



Paraformaldehyde-fixed, paraffin embedded (Rat liver), 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 (HMGB1) Polyclonal Antibody, Unconjugated (orb500823) at 1:500 overnight at 4°C, followed by a conjugated secondary for 20 minutes and DAB staining.

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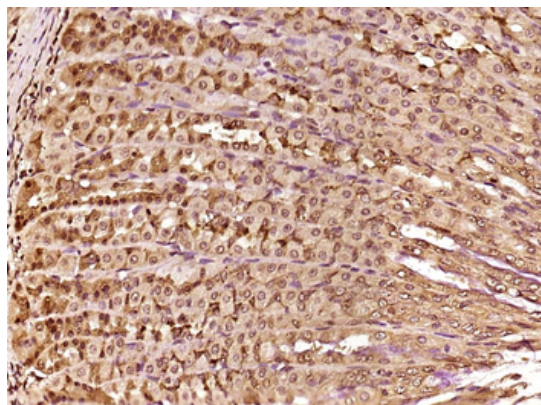
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Paraformaldehyde-fixed, paraffin embedded (Rat stomach), Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15 min, Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes, Blocking buffer (normal goat serum) at 37°C for 30 min, Antibody incubation with (HMGB1) Polyclonal Antibody, Unconjugated (orb500823) at 1:500 overnight at 4°C, followed by a conjugated secondary for 20 minutes and DAB staining.

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