

Product Datasheet

HMGB1 Rabbit Polyclonal Antibody (orb389479)

Catalog Number	orb389479
Category	Antibodies
Description	HMGB1 Rabbit Polyclonal Antibody
Target	High mobility group protein B1
Clonality	Polyclonal
Species/Host	Rabbit
Isotype	Rabbit IgG
Conjugation	Unconjugated
Reactivity	Human, Mouse, Rat
Form/Appearance	Lyophilized
Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 µg/ml.
Buffer/Preservatives	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na ₂ HPO ₄ , 0.05mg NaN ₃ .
Reconstitution	Add 0.2ml of distilled water will yield a concentration of 500ug/ml.
Purification	Immunogen affinity purified.
Immunogen	A synthetic peptide corresponding to a sequence at the C-terminus of human HMGB1, identical to the related mouse and rat sequences.
UniProt ID	P09429
MW	25 kDa

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

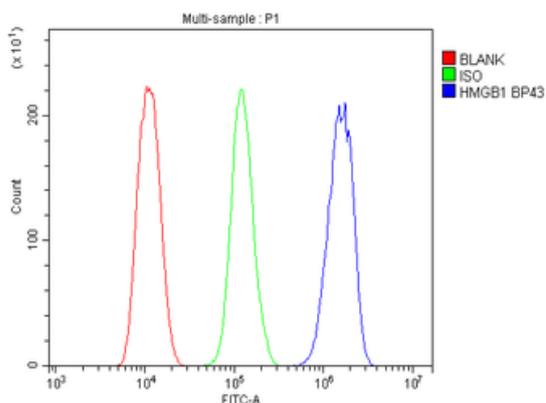
Email: info@biorbyt.com, support@biorbyt.com
Phone: [+44 \(0\)1223 859353](tel:+44(0)1223859353) | Fax: [+1 \(415\) 651-8558](tel:+1(415)651-8558)

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)

Tested applications	FC, ICC, IF, IHC, WB
Dilution range	Immunohistochemistry (Paraffin-embedded Section), 0.5-1µg/ml, Human, Mouse, Rat Western blot, 0.1-0.5µg/ml, Human, Mouse, Rat Immunocytochemistry/Immunofluorescence, 2µg/ml, Human Flow Cytometry (Fixed), 1-3µg/1x10 ⁶ cells, Human
Specificity	No cross reactivity with other proteins.
Cross Reactivity	No cross-reactivity with other proteins
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.



Flow Cytometry analysis of THP-1 cells using anti-HMGB1 antibody. Overlay histogram showing THP-1 cells (Blue line). To facilitate intracellular staining, cells were fixed with 4% paraformaldehyde and permeabilized with permeabilization buffer. The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-HMGB1 Antibody (1 µg/1x10⁶ cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (5-10 µg/1x10⁶ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1 µg/1x10⁶) used under the same conditions. Unlabelled sample without incubation with primary antibody and secondary antibody (Red line) was used as a blank control.

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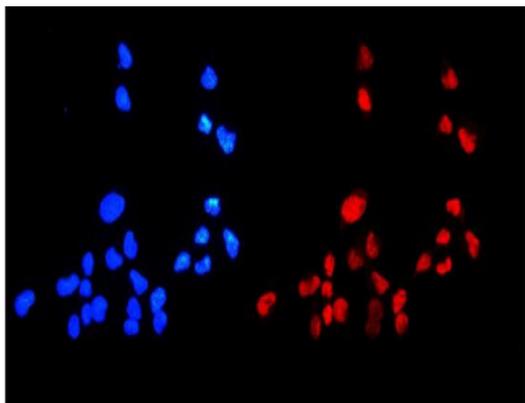
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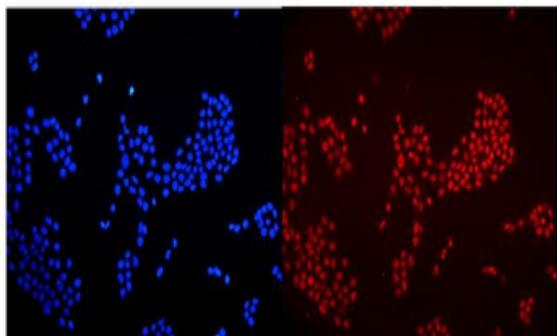
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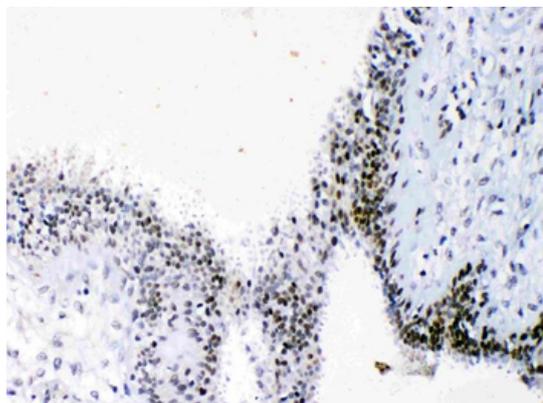
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IF analysis of HMGB1 using anti-HMGB1 antibody. HMGB1 was detected in immunocytochemical section of U2OS cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 2 $\mu\text{g}/\text{mL}$ rabbit anti-HMGB1 Antibody overnight at 4°C. DyLight®594 Conjugated Goat Anti-Rabbit IgG was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.



IF analysis of HMGB1 using anti-HMGB1 antibody. HMGB1 was detected in immunocytochemical section of A431 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 2 $\mu\text{g}/\text{mL}$ rabbit anti-HMGB1 Antibody overnight at 4°C. DyLight®594 Conjugated Goat Anti-Rabbit IgG was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.



IHC analysis of HMGB1 using anti-HMGB1 antibody. HMGB1 was detected in paraffin-embedded section of human mammary cancer tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 $\mu\text{g}/\text{ml}$ rabbit anti-HMGB1 Antibody overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) with DAB as the chromogen.

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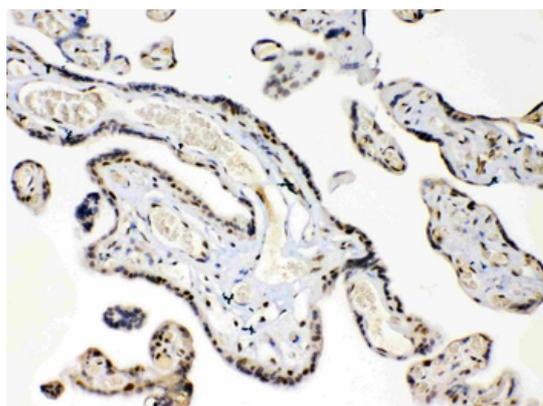
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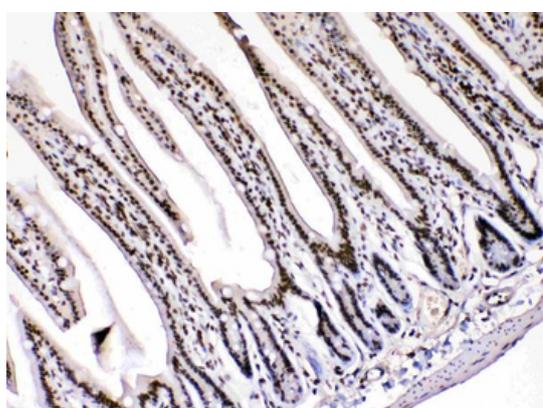
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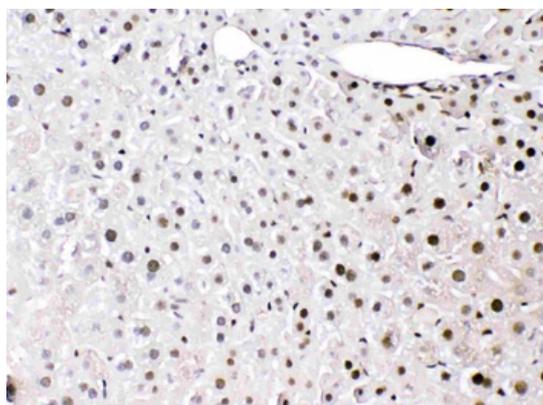
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IHC analysis of HMGB1 using anti-HMGB1 antibody. HMGB1 was detected in paraffin-embedded section of human placenta tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 μ g/ml rabbit anti-HMGB1 Antibody overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) with DAB as the chromogen.



IHC analysis of HMGB1 using anti-HMGB1 antibody. HMGB1 was detected in paraffin-embedded section of mouse intestine tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 μ g/ml rabbit anti-HMGB1 Antibody overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) with DAB as the chromogen.



IHC analysis of HMGB1 using anti-HMGB1 antibody. HMGB1 was detected in paraffin-embedded section of mouse liver tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 μ g/ml rabbit anti-HMGB1 Antibody overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) with DAB as the chromogen.

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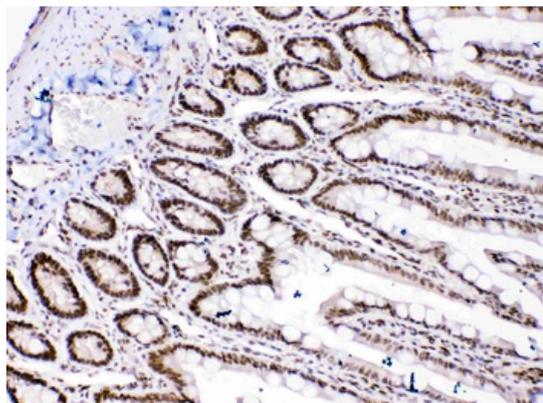
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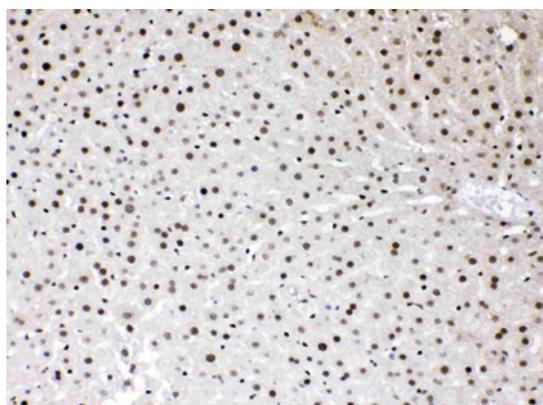
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IHC analysis of HMGB1 using anti-HMGB1 antibody. HMGB1 was detected in paraffin-embedded section of rat intestine tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 μ g/ml rabbit anti-HMGB1 Antibody overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) with DAB as the chromogen.



IHC analysis of HMGB1 using anti-HMGB1 antibody. HMGB1 was detected in paraffin-embedded section of rat liver tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 μ g/ml rabbit anti-HMGB1 Antibody overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) with DAB as the chromogen.

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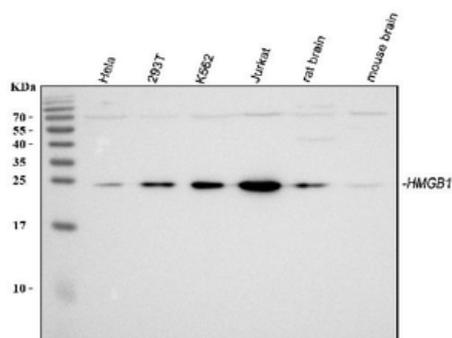
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Western blot analysis of HMGB1 using anti-HMGB1 antibody. Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions. Lane 1: human Hela whole cell lysates, Lane 2: human 293T whole cell lysates, Lane 3: human K562 whole cell lysates, Lane 4: human Jurkat whole cell lysates, Lane 5: rat brain tissue lysates, Lane 6: mouse brain tissue lysates. After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA for 50-90 minutes. Blocked the membrane with 5% non-fat milk/TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-HMGB1 antigen affinity purified polyclonal antibody at 0.5 μ g/mL overnight at 4°C, then washed with TBS-0.1% Tween 3 times with 5 minutes each and probed with a goat anti-rabbit IgG-HRP secondary antibody at a dilution of 1:5000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit with Tanon 5200 system. A specific band was detected for HMGB1 at approximately 25 kDa. The expected band size for HMGB1 is at 25 kDa.

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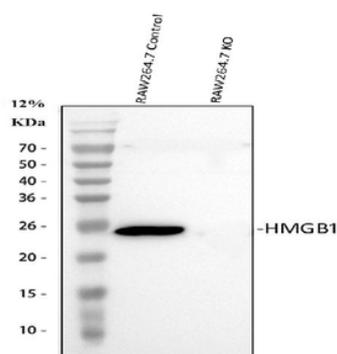
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