

Anti-HMGB1 Antibody Picoband® (monoclonal, 5H3)

Catalog Number: M00066-2

About HMGB1

High mobility group box 1 protein, also known as high-mobility group protein 1 (HMG-1) and amphoterin, is a protein that in humans is encoded by the HMGB1 gene. This gene encodes a protein that belongs to the High Mobility Group-box superfamily. The encoded non-histone, nuclear DNA-binding protein regulates transcription, and is involved in organization of DNA. This protein plays a role in several cellular processes, including inflammation, cell differentiation and tumor cell migration. Multiple pseudogenes of this gene have been identified. Alternative splicing results in multiple transcript variants that encode the same protein.

Overview

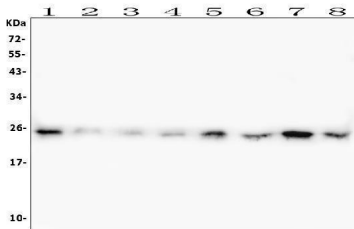
Product Name	Anti-HMGB1 Antibody Picoband® (monoclonal, 5H3)
Reactive Species	Human, Monkey, Mouse, Rat
Description	Boster Bio Anti-HMGB1 Antibody Picoband® (monoclonal, 5H3) catalog # M00066-2. Tested in Flow Cytometry, IHC, WB applications. This antibody reacts with Human, Monkey, Mouse, Rat. The brand Picoband indicates this is a premium antibody that guarantees superior quality, high affinity, and strong signals with minimal background in Western blot applications. Only our best-performing antibodies are designated as Picoband, ensuring unmatched performance.
Application	Flow Cytometry, IHC, WB
Clonality	Monoclonal 5H3
Formulation	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na ₂ HPO ₄ , 0.05mg NaN ₃ .
Storage Instructions	Store at -20°C for one year from date of receipt. After reconstitution, at 4°C for one month. It can also be aliquotted and stored frozen at -20°C for six months. Avoid repeated freeze-thaw cycles.
Host	Mouse
Uniprot ID	P09429

Technical Details

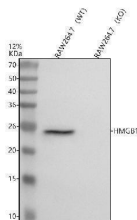
Immunogen	A synthetic peptide corresponding to a sequence at the C-terminus of human HMGB1, identical to the related mouse and rat sequences.
Recommended Detection Systems	Boster recommends Enhanced Chemiluminescent Kit with anti-Mouse IgG (EK1001) for Western blot, and HRP Conjugated anti-Mouse IgG Super Vision Assay Kit (SV0001-1) for IHC(P).
Cross Reactivity	No cross-reactivity with other proteins.
Isotype	Mouse IgG2b
Form	Lyophilized

Concentration	0
Suggested Dilutions	Western blot, 0.1-0.5ug/ml, Human, Mouse, Rat, Monkey Immunohistochemistry (Paraffin-embedded Section), 0.5-1ug/ml, Human, Mouse, Rat Flow Cytometry (Fixed), 1-3ug/1x10 ⁶ cells, Human

Anti-HMGB1 Antibody Picoband® (monoclonal, 5H3) (M00066-2) Images

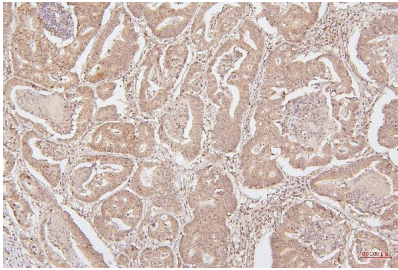


Western blot analysis of HMGB1 using anti-HMGB1 antibody (M00066-2). Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions. Lane 1: human HepG2 whole cell lysates Lane 2: human CCRF-CEM whole cell lysates Lane 3: monkey COS-7 whole cell lysates Lane 4: human SW620 whole cell lysates Lane 5: human THP-1 whole cell lysates Lane 6: rat PC-12 whole cell lysates Lane 7: rat RH35 whole cell lysates Lane 8: mouse NIH/3T3 whole cell lysates After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA for 50-90 minutes. Blocked the membrane with 5% Non-fat Milk/ TBS for 1.5 hour at RT. The membrane was incubated with mouse anti-HMGB1 antigen affinity purified monoclonal antibody (Catalog # M00066-2) at 0.5 ug/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-mouse IgG-HRP secondary antibody at a dilution of 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1001) with Tanon 5200 system. A specific band was detected for HMGB1 at approximately 25KD. The expected band size for HMGB1 is at 25KD.

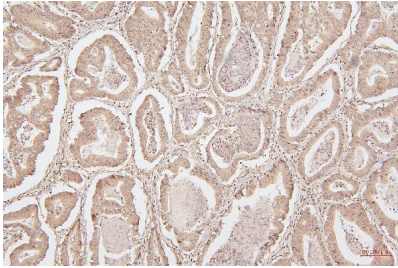


Western blot analysis of HMGB1 using anti-HMGB1 antibody (M00066-2). Electrophoresis was performed on a 12% SDS-PAGE gel at 80V (Stacking gel) / 120V (Resolving gel) for 2 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions. Lane 1: mouse RAW264.7- WT whole cell lysates, Lane 2: mouse RAW264.7-Hmgb1 KO whole cell 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. Then the membrane was incubated with mouse anti-HMGB1 antigen affinity purified monoclonal antibody (M00066-2) at 0.5 ug/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-mouse IgG-HRP secondary antibody (Catalog # BA1051) at a dilution of 1:5000 for 1.5 hour at RT. The signal is developed using an ECL Plus Western Blotting Substrate (Catalog # AR1196-200) 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.

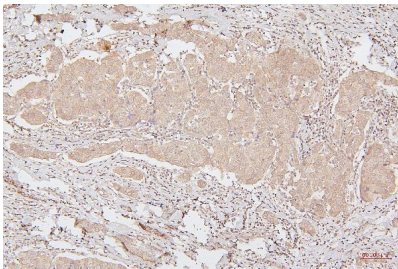
IHC analysis of HMGB1 using anti-HMGB1 antibody (M00066-2). HMGB1 was detected in paraffin-embedded section of human intestinal cancer tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was



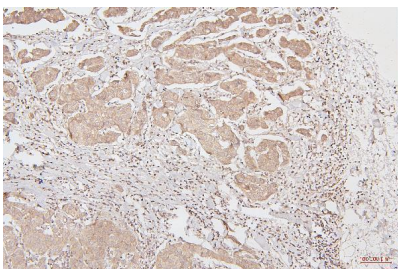
then incubated with 1ug/ml mouse anti-HMGB1 Antibody (M00066-2) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.



IHC analysis of HMGB1 using anti-HMGB1 antibody (M00066-2). HMGB1 was detected in paraffin-embedded section of human intestinal 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 1ug/ml mouse anti-HMGB1 Antibody (M00066-2) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

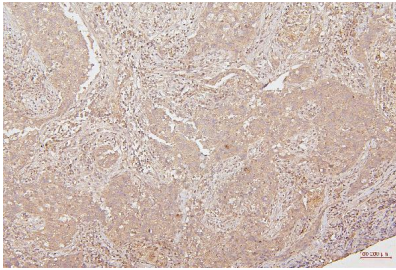


IHC analysis of HMGB1 using anti-HMGB1 antibody (M00066-2). HMGB1 was detected in paraffin-embedded section of human mammary cancer tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml mouse anti-HMGB1 Antibody (M00066-2) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

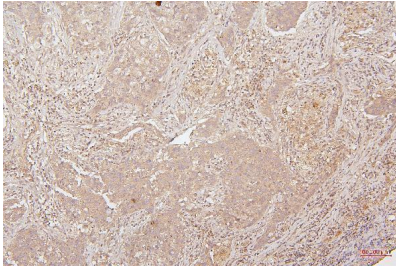


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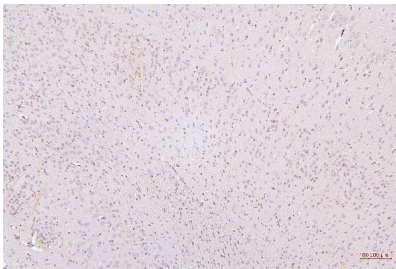
IHC analysis of HMGB1 using anti-HMGB1 antibody (M00066-2). HMGB1 was detected in paraffin-embedded section of human lung cancer tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml mouse anti-HMGB1 Antibody



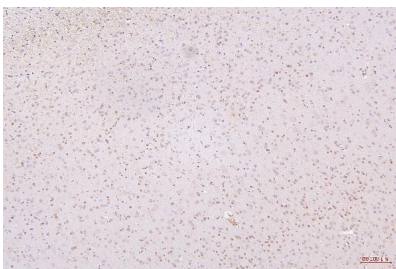
(M00066-2) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.



IHC analysis of HMGB1 using anti-HMGB1 antibody (M00066-2). HMGB1 was detected in paraffin-embedded section of human lung 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 1ug/ml mouse anti-HMGB1 Antibody (M00066-2) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

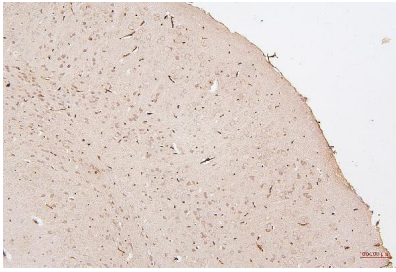


IHC analysis of HMGB1 using anti-HMGB1 antibody (M00066-2). HMGB1 was detected in paraffin-embedded section of rat brain tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml mouse anti-HMGB1 Antibody (M00066-2) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

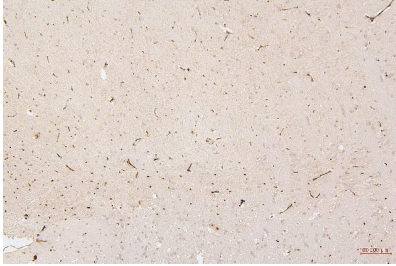


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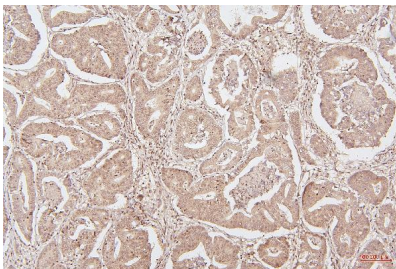
(M00066-2) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.



IHC analysis of HMGB1 using anti-HMGB1 antibody (M00066-2). HMGB1 was detected in paraffin-embedded section of mouse brain 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 1ug/ml mouse anti-HMGB1 Antibody (M00066-2) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

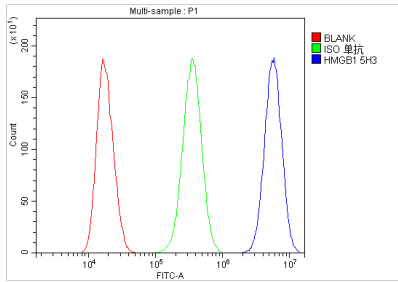


IHC analysis of HMGB1 using anti-HMGB1 antibody (M00066-2). HMGB1 was detected in paraffin-embedded section of mouse brain tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml mouse anti-HMGB1 Antibody (M00066-2) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.



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Flow Cytometry analysis of SiHa cells using anti-HMGB1 antibody (M00066-2). Overlay histogram showing SiHa cells stained with M00066-2 (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 mouse anti-HMGB1 Antibody (M00066-2,1ug/1x10⁶



cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10ug/1x10⁶ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1ug/1x10⁶) used under the same conditions. Unlabelled sample without incubation with primary antibody and secondary antibody (Red line) was used as a blank control.

7 Publications Citing This Product

1. PubMed ID: 10.1016/j.phymed.2014.10.011, Total extract of Yupingfeng attenuates bleomycin-induced pulmonary fibrosis in rats
2. PubMed ID: 32417325, Wang F, Ji S, Wang M, Liu L, Li Q, Jiang F, Cen J, Ji B. HMGB1 promoted P-glycoprotein at the blood-brain barrier in MCAO rats via TLR4/NF-kappaB signaling pathway. Eur J Pharmacol. 2020 Aug 5; 880:173189. doi:10.1016/j.ejphar.2020.173189. Epub 2020 May 15. PMID:32417325.
3. PubMed ID: 31933513, Wen Y, Sun HY, Tan Z, Liu RH, Huang SQ, Chen GY, Qi H, Tang LJ. Abdominal paracentesis drainage ameliorates myocardial injury in severe experimental pancreatitis rats through suppressing oxidative stress. World J Gastroenterol. 2020 Jan 7; 26(1):35-54. doi:10.3748/wj

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