10320 Camino Santa Fe, Suite G
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Tel: 858.875.1900 Fax: 858.875.1999

Anti-HMGB1/Hmg 1 Rabbit Monoclonal Antibody

Catalog # ABO13578

Specification

Anti-HMGB1/Hmg 1 Rabbit Monoclonal Antibody - Product Information

Application	**WB, IHC, IF, ICC, FC**
Primary Accession	P09429
Host	**Rabbit**
Isotype	**Rabbit IgG**
Reactivity	**Rat, Human, Mouse**
Clonality	**Monoclonal**
Format	**Liquid**

Description
Anti-HMGB1/Hmg 1 Rabbit Monoclonal Antibody . Tested in WB, IHC, ICC/IF, Flow Cytometry applications. This antibody reacts with Human, Mouse, Rat.

Anti-HMGB1/Hmg 1 Rabbit Monoclonal Antibody - Additional Information

Gene ID 3146

Other Names
High mobility group protein B1, High mobility group protein 1, HMG-1, HMGB1 (HGNC:4983), HMG1

Calculated MW
24894 MW KDa

Application Details
WB 1:1000-1:2000
IHC 1:50-1:100
ICC/IF 1:50-1:100
FC 1:30

Subcellular Localization
Nucleus. Chromosome. Cytoplasm. Secreted. Cell membrane ; Peripheral membrane protein ; Extracellular side. Endosome. Endoplasmic reticulum-Golgi intermediate compartment. In basal state predominantly nuclear. Shuttles between the cytoplasm and the nucleus (PubMed:12231511, PubMed:17114460). Translocates from the nucleus to the cytoplasm upon autophagy stimulation (PubMed:20819940). Release from macrophages in the extracellular milieu requires the activation of NLRC4 or NLRP3 inflammasomes (By similarity). Passively released to the extracellular milieu from necrotic cells by diffusion, involving the fully reduced HGMB1 which subsequently gets oxidized (PubMed:19811284). Also released from apoptic cells (PubMed:16855214, PubMed:18631454). Active secretion from a variety of immune and non-immune cells such as macrophages, monocytes, neutrophils, dendritic cells and natural killer cells in response to various stimuli such as LPS and cytokines involves a nonconventional secretory process via secretory lysosomes (PubMed:12231511, PubMed:14532127, PubMed:15944249). Secreted by plasma cells in response to LPS (By similarity). Found on the surface of activated platelets (PubMed:11154118)..

Tissue Specificity
Ubiquituous. Expressed in platelets (PubMed:11154118)..

Contents
Rabbit IgG in phosphate buffered saline, pH 7.4, 150mM NaCl, 0.02% sodium azide and 50% glycerol, 0.4-0.5mg/ml BSA.

Immunogen
A synthesized peptide derived from human HMGB1

Purification
Affinity-chromatography

Storage	**Store at -20°C for one year. For short term storage and frequent use, store at 4°C for up to one month. Avoid repeated freeze-thaw cycles.**

Anti-HMGB1/Hmg 1 Rabbit Monoclonal Antibody - Protein Information

Name HMGB1 (HGNC:4983)

Synonyms HMG1

Function
Multifunctional redox sensitive protein with various roles in different cellular compartments. In the nucleus is one of the major chromatin-associated non-histone proteins and acts as a DNA chaperone involved in replication, transcription, chromatin remodeling, V(D)J recombination, DNA repair and genome stability (PubMed:33147444). Proposed to be an universal biosensor for nucleic acids. Promotes host inflammatory response to sterile and infectious signals and is involved in the coordination and integration of innate and adaptive immune responses. In the cytoplasm functions as a sensor and/or chaperone for immunogenic nucleic acids implicating the activation of TLR9-mediated immune responses, and mediates autophagy. Acts as a danger-associated molecular pattern (DAMP) molecule that amplifies immune responses during tissue injury (PubMed:27362237). Released to the extracellular environment can bind DNA, nucleosomes, IL-1 beta, CXCL12, AGER isoform 2/sRAGE, lipopolysaccharide (LPS) and lipoteichoic acid (LTA), and activates cells through engagement of multiple surface receptors (PubMed:34743181). In the extracellular compartment fully reduced HMGB1 (released by necrosis) acts as a chemokine, disulfide HMGB1 (actively secreted) as a cytokine, and sulfonyl HMGB1 (released from apoptotic cells) promotes immunological tolerance (PubMed:23446148, PubMed:23519706, PubMed:23994764, PubMed:25048472). Has proangiogdenic activity (By similarity). May be involved in platelet activation (By similarity). Binds to phosphatidylserine and phosphatidylethanolamide (By similarity). Bound to RAGE mediates signaling for neuronal outgrowth (By similarity). May play a role in accumulation of expanded polyglutamine (polyQ) proteins such as huntingtin (HTT) or TBP (PubMed:23303669, PubMed:25549101).

Cellular Location
Nucleus. Chromosome {ECO:0000250|UniProtKB:P10103, ECO:0000250|UniProtKB:P63159, ECO:0000305}. Cytoplasm. Secreted {ECO:0000250|UniProtKB:P63158, ECO:0000269|PubMed:12231511, ECO:0000269|PubMed:14532127, ECO:0000269|PubMed:15944249, ECO:0000269|PubMed:19811284,

ECO:0000269|PubMed:22869893, ECO:0000269|PubMed:33147444}. Cell membrane {ECO:0000250|UniProtKB:P63158, ECO:0000250|UniProtKB:P63159, ECO:0000269|PubMed:11154118}; Peripheral membrane protein {ECO:0000250|UniProtKB:P63158, ECO:0000250|UniProtKB:P63159, ECO:0000269|PubMed:11154118}; Extracellular side {ECO:0000250|UniProtKB:P63158, ECO:0000250|UniProtKB:P63159, ECO:0000269|PubMed:11154118}. Endosome {ECO:0000250|UniProtKB:P63158} Endoplasmic reticulum-Golgi intermediate compartment {ECO:0000250|UniProtKB:P63158}. Note=In basal state predominantly nuclear. Shuttles between the cytoplasm and the nucleus (PubMed:12231511, PubMed:17114460). Translocates from the nucleus to the cytoplasm upon autophagy stimulation (PubMed:20819940). Release from macrophages in the extracellular milieu requires the activation of NLRC4 or NLRP3 inflammasomes (By similarity). Passively released to the extracellular milieu from necrotic cells by diffusion, involving the fully reduced HGMB1 which subsequently gets oxidized (PubMed:19811284) Also released from apoptotic cells (PubMed:16855214, PubMed:18631454) Active secretion from a variety of immune and non-immune cells such as macrophages, monocytes, neutrophils, dendritic cells and natural killer cells in response to various stimuli such as LPS and cytokines involves a nonconventional secretory process via secretory lysosomes (PubMed:12231511, PubMed:14532127, PubMed:15944249). Secreted by plasma cells in response to LPS (By similarity). Found on the surface of activated platelets (PubMed:11154118). An increased chromatin association is observed when associated with the adenovirus protein pVII (PubMed:27362237). {ECO:0000250|UniProtKB:P63158, ECO:0000269|PubMed:11154118, ECO:0000269|PubMed:12231511, ECO:0000269|PubMed:14532127, ECO:0000269|PubMed:15944249, ECO:0000269|PubMed:16855214, ECO:0000269|PubMed:17114460, ECO:0000269|PubMed:18631454, ECO:0000269|PubMed:19811284, ECO:0000269|PubMed:20819940, ECO:0000269|PubMed:27362237, ECO:0000305|PubMed:20123072}

Tissue Location
Ubiquitous. Expressed in platelets (PubMed:11154118).

Anti-HMGB1/Hmg 1 Rabbit Monoclonal Antibody - Protocols

Provided below are standard protocols that you may find useful for product applications.

- Western Blot
- Blocking Peptides
- Dot Blot
- Immunohistochemistry
- Immunofluorescence
- Immunoprecipitation
- Flow Cytomety
- Cell Culture

Anti-HMGB1/Hmg 1 Rabbit Monoclonal Antibody - Images

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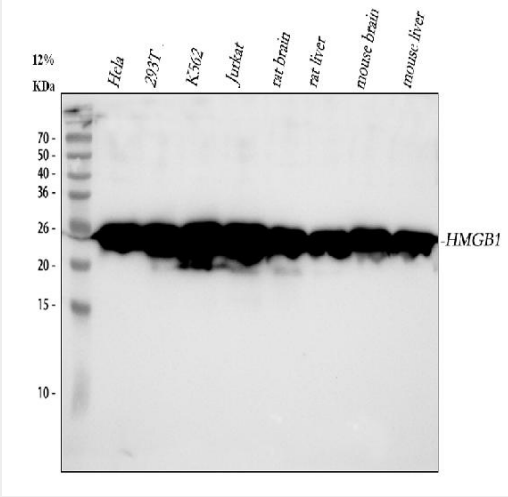

Figure 1. Western blot analysis of HMGB1 using anti-HMGB1 antibody (M00066-1).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions.
Lane 1: human Hela whole 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: rat liver tissue lysates,
Lane 7: mouse brain tissue lysates,
Lane 8: mouse liver 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 monoclonal antibody (Catalog # M00066-1) at 1:1000 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 1:1000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1002) 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.

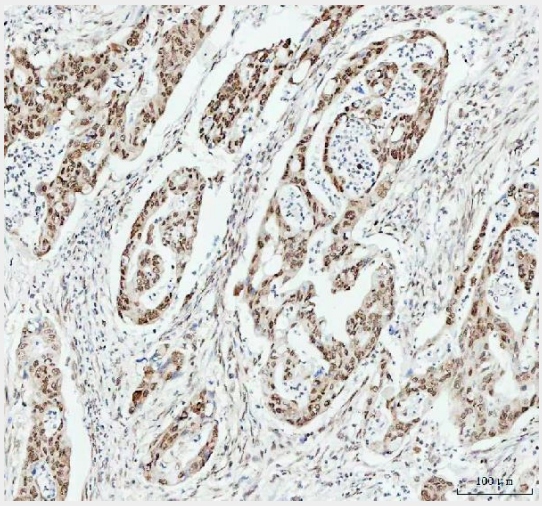

Figure 2. IHC analysis of HMGB1 using anti-HMGB1 antibody (M00066-1).
HMGB1 was detected in a paraffin-embedded section of human colorectal adenocarcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution).

The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1:50 rabbit anti-HMGB1 Antibody (M00066-1) overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit (Catalog # SV0002) with DAB as the chromogen.

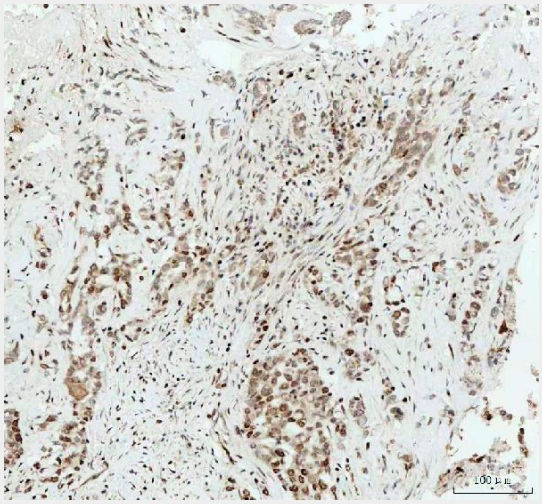

Figure 3. IHC analysis of HMGB1 using anti-HMGB1 antibody (M00066-1).
HMGB1 was detected in a paraffin-embedded section of human lung adenocarcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1:50 rabbit anti-HMGB1 Antibody (M00066-1) overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit (Catalog # SV0002) with DAB as the chromogen.

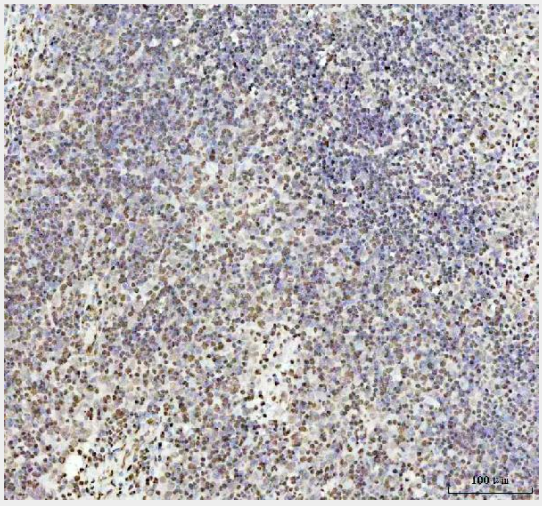

Figure 4. IHC analysis of HMGB1 using anti-HMGB1 antibody (M00066-1).
HMGB1 was detected in a paraffin-embedded section of human spleen tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1:50 rabbit anti-HMGB1 Antibody (M00066-1) overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit (Catalog # SV0002) with DAB as the chromogen.

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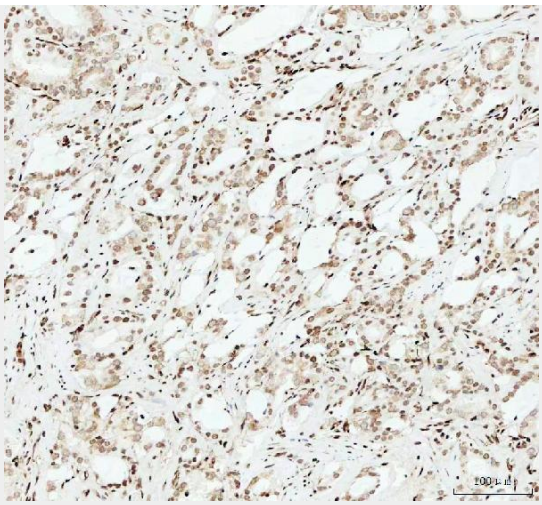


Figure 5. IHC analysis of HMGB1 using anti-HMGB1 antibody (M00066-1).
HMGB1 was detected in a paraffin-embedded section of human prostatic acinar adenocarcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1:50 rabbit anti-HMGB1 Antibody (M00066-1) overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit (Catalog # SV0002) with DAB as the chromogen.

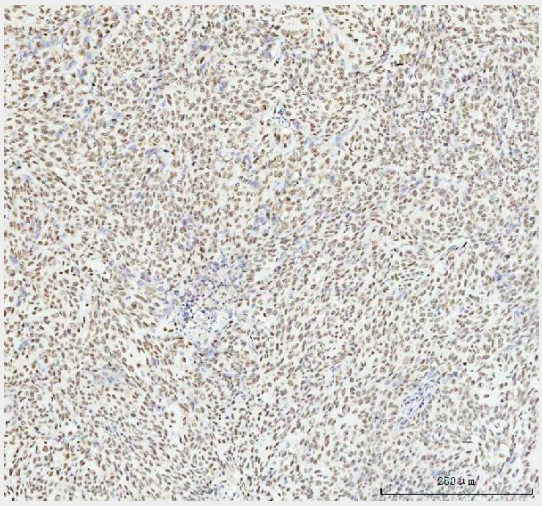


Figure 6. IHC analysis of HMGB1 using anti-HMGB1 antibody (M00066-1).
HMGB1 was detected in a paraffin-embedded section of human cervical squamous carcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1:50 rabbit anti-HMGB1 Antibody (M00066-1) overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit (Catalog # SV0002) with DAB as the chromogen.

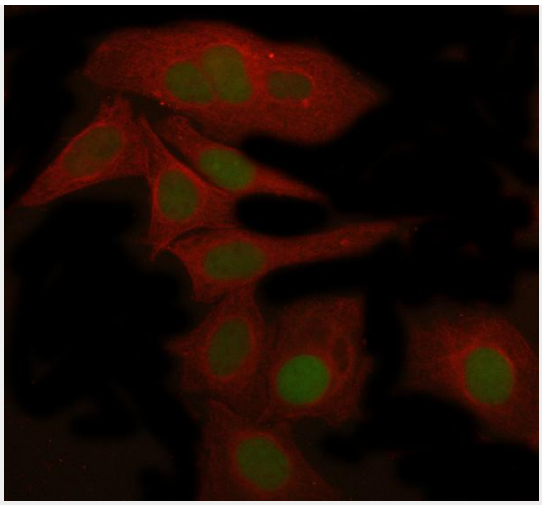
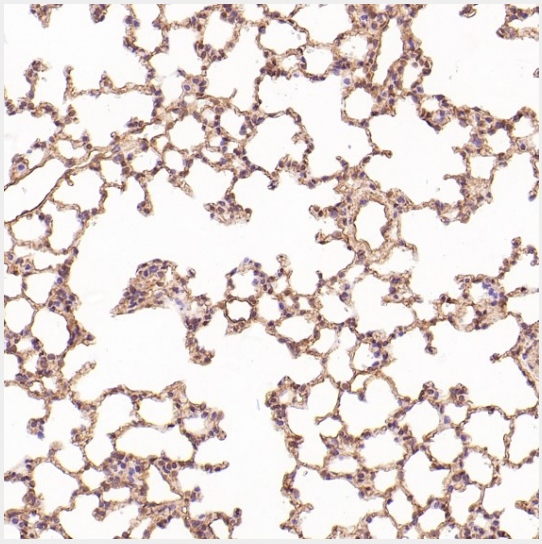


Figure 7. IF analysis of HMGB1 using anti-HMGB1 antibody (M00066-1) and anti-Beta Tubulin antibody (M01857-3).
HMGB1 was detected in immunocytochemical section of Hela cell. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated at 1:50 with rabbit anti-HMGB1 Antibody (M00066-1) and mouse anti-Beta Tubulin antibody (M01857-3) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Rabbit IgG (BA1127) and Cy3 Conjugated Goat Anti-Mouse IgG (BA1031) were used as secondary antibody at 1:500 dilution and incubated for 30 minutes at 37°C. Visualize using a fluorescence microscope and filter sets appropriate for the label used.



Immunohistochemical analysis of paraffin-embedded Rat liver, using the Antibody at 1:300 dilution.

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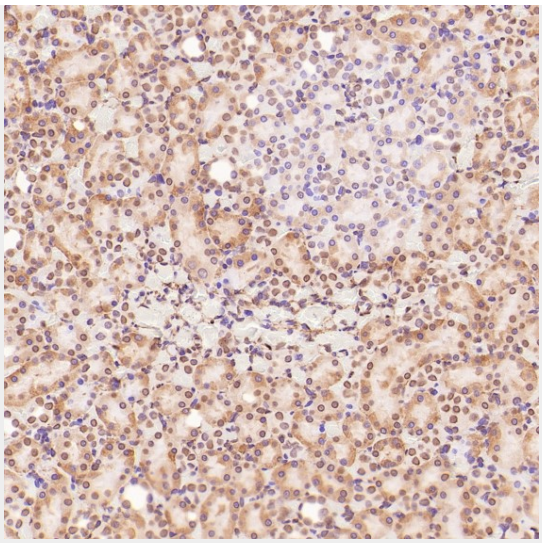
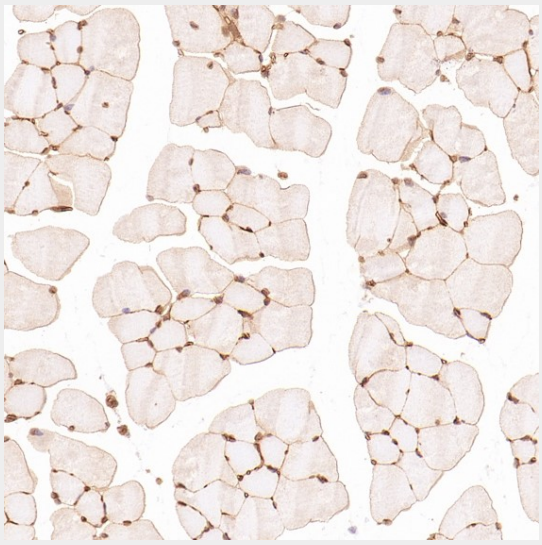


Immunohistochemical analysis of paraffin-embedded Rat kidney, using the Antibody at 1:300 dilution.



Immunohistochemical analysis of paraffin-embedded Mouse skeletal muscle - gastrocnemius , using the Antibody at 1:300 dilution.