10320 Camino Santa Fe, Suite G
San Diego, CA 92121
Tel: 858.875.1900 Fax: 858.875.1999

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

Catalog # ABO14876

Specification

Anti-HMGB1 Antibody Picoband™ (monoclonal, 5H3) - Product Information

Application	**WB, IHC, FC**
Primary Accession	P09429
Host	**Mouse**
Isotype	**Mouse IgG2b**
Reactivity	**Rat, Human, Mouse, Monkey**
Clonality	**Monoclonal**
Format	**Lyophilized**

Description
Anti-HMGB1 Antibody Picoband™ (monoclonal, 5H3) . Tested in Flow Cytometry, IHC, WB applications. This antibody reacts with Human, Monkey, Mouse, Rat.

Reconstitution
Add 0.2ml of distilled water will yield a concentration of 500 µg/ml.

Anti-HMGB1 Antibody Picoband™ (monoclonal, 5H3) - 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
25 kDa KDa

Application Details
Western blot, 0.1-0.5 µg/ml, Human, Mouse, Rat, Monkey
 Immunohistochemistry (Paraffin-embedded Section), 0.5-1 µg/ml, Human, Mouse, Rat, By Heat
 Flow Cytometry, 1-3 µg/1x10^6 cells, Human

Subcellular Localization
Nucleus. Endosome. Secreted. Cell membrane. Peripheral membrane protein. Extracellular side. Chromosome. Cytoplasm. Endoplasmic reticulum-Golgi intermediate compartment.

Tissue Specificity
Ubiquituous. Expressed in platelets.

Contents
Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na₂HPO₄, 0.05mg NaN₃.

Immunogen
A synthetic peptide corresponding to a sequence at the C-terminus of human HMGB1, identical to

the related mouse and rat sequences.

Cross Reactivity
No cross-reactivity with other proteins.

Storage

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.

Anti-HMGB1 Antibody Picoband™ (monoclonal, 5H3) - 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 Antibody Picoband™ (monoclonal, 5H3) - 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 Antibody Picoband™ (monoclonal, 5H3) - Images

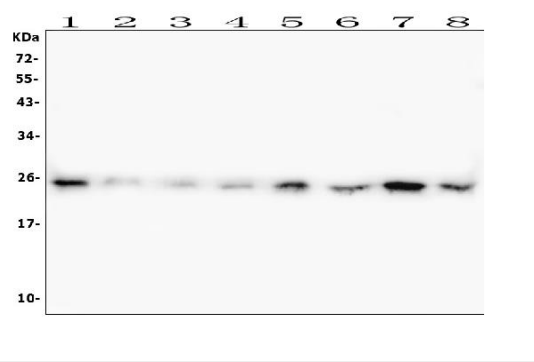

Figure 1. Western blot analysis of HMGB1 using anti-HMGB1 antibody (M00066-2).

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 µg/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.

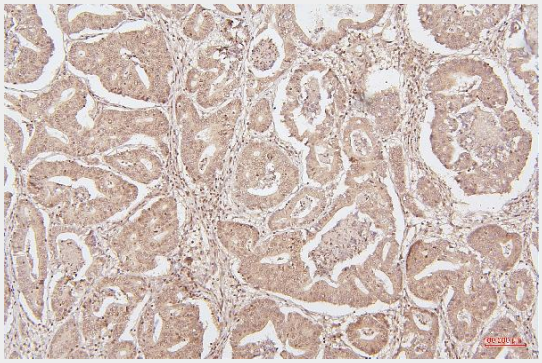

Figure 2. IHC analysis of HMGB1 using anti-HMGB1 antibody (M00066-2).
HMGB1 was detected in paraffin-embedded section of human intestinal cancer tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/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 Strepavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

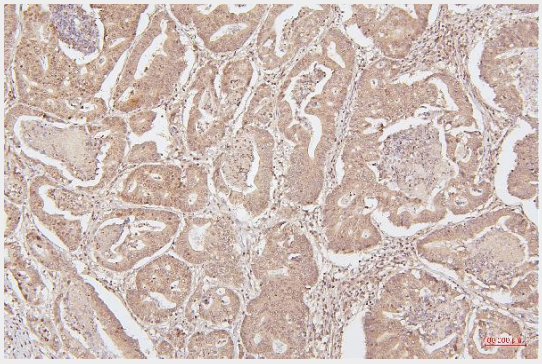

Figure 3. IHC analysis of HMGB1 using anti-HMGB1 antibody (M00066-2).
HMGB1 was detected in paraffin-embedded section of human intestinal cancer tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated

with 1 µg/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 Strepavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

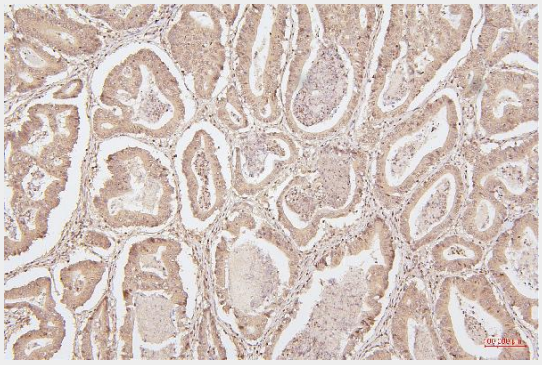

Figure 4. IHC analysis of HMGB1 using anti-HMGB1 antibody (M00066-2).
HMGB1 was detected in paraffin-embedded section of human intestinal cancer tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/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 Strepavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

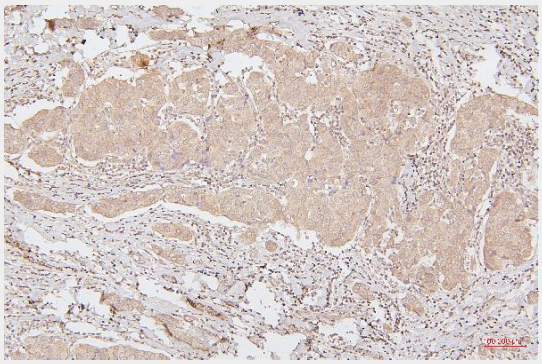

Figure 5. IHC analysis of HMGB1 using anti-HMGB1 antibody (M00066-2).
HMGB1 was detected in paraffin-embedded section of human mammary cancer tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/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 Strepavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

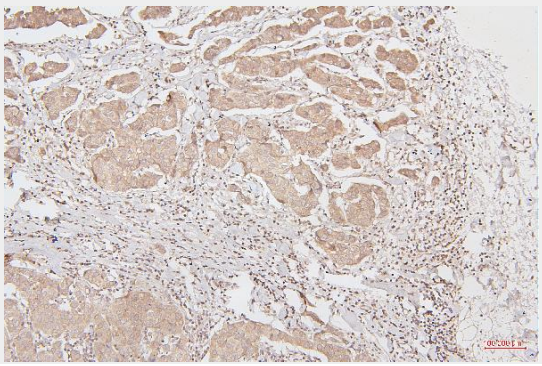

Figure 6. IHC analysis of HMGB1 using anti-HMGB1 antibody (M00066-2).
HMGB1 was detected in paraffin-embedded section of human mammary cancer tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/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 Strepavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

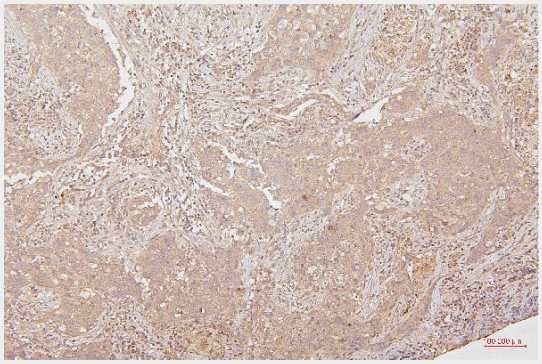

Figure 7. IHC analysis of HMGB1 using anti-HMGB1 antibody (M00066-2).
HMGB1 was detected in paraffin-embedded section of human lung cancer tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/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 Strepavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

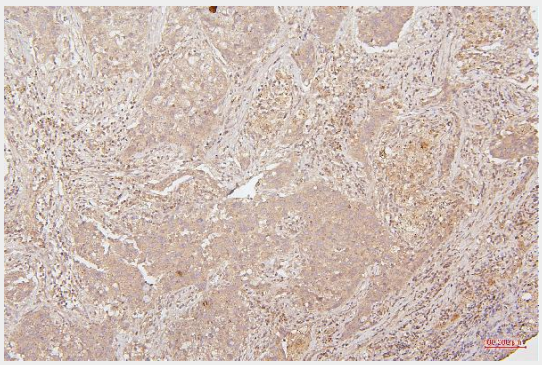

Figure 8. IHC analysis of HMGB1 using anti-HMGB1 antibody (M00066-2).
HMGB1 was detected in paraffin-embedded section of human lung cancer tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/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 Strepavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

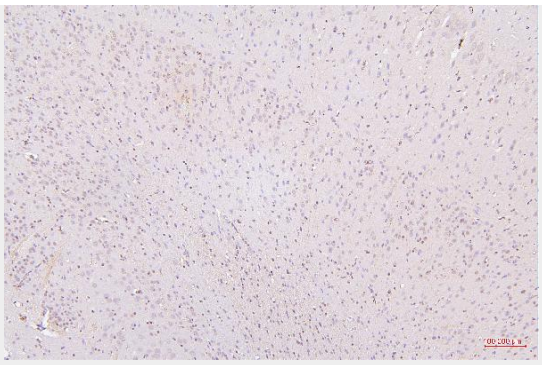

Figure 9. IHC analysis of HMGB1 using anti-HMGB1 antibody (M00066-2).
HMGB1 was detected in paraffin-embedded section of rat brain tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/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 Strepavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

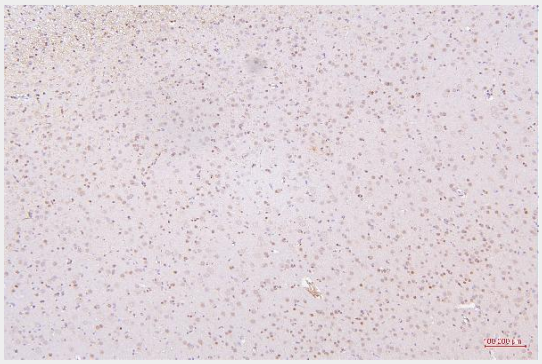

Figure 10. IHC analysis of HMGB1 using anti-HMGB1 antibody (M00066-2).
HMGB1 was detected in paraffin-embedded section of rat brain tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/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 Strepavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

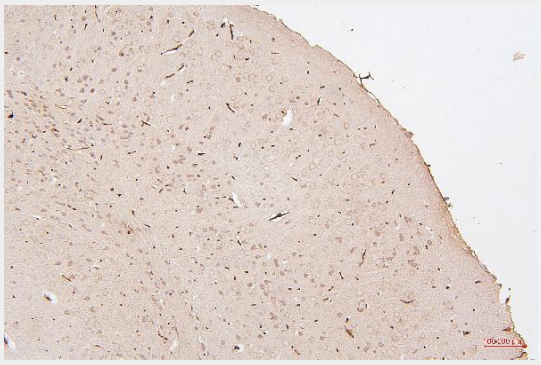

Figure 11. IHC analysis of HMGB1 using anti-HMGB1 antibody (M00066-2).
HMGB1 was detected in paraffin-embedded section of mouse brain tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The

tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/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 Strepavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

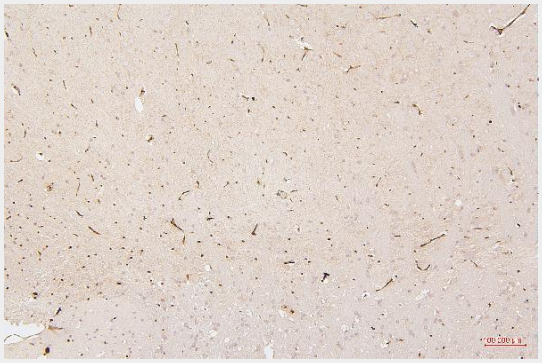

Figure 12. IHC analysis of HMGB1 using anti-HMGB1 antibody (M00066-2).
HMGB1 was detected in paraffin-embedded section of mouse brain tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/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 Strepavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

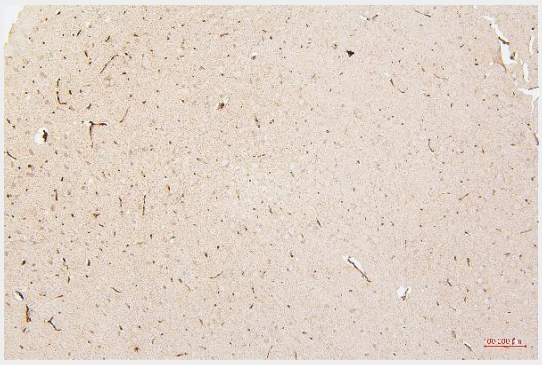

Figure 13. IHC analysis of HMGB1 using anti-HMGB1 antibody (M00066-2).
HMGB1 was detected in paraffin-embedded section of mouse brain tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/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 Strepavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

Anti-HMGB1 Antibody Picoband™ (monoclonal, 5H3) - Background

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.