**Product datasheet**

**Anti-HMGB1 antibody - ChIP Grade ab18256**

⭐⭐⭐⭐⭐ 87 Abreviews  251 References  12 Images

**Overview**

<table>
<thead>
<tr>
<th><strong>Product name</strong></th>
<th>Anti-HMGB1 antibody - ChIP Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Description</strong></td>
<td>Rabbit polyclonal to HMGB1 - ChIP Grade</td>
</tr>
<tr>
<td><strong>Host species</strong></td>
<td>Rabbit</td>
</tr>
<tr>
<td><strong>Tested applications</strong></td>
<td>Suitable for: IHC-FoFr, ICC/IF, IHC-P, ChIP, IHC-Fr, ICC, ELISA, IP, WB</td>
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<tr>
<td><strong>Species reactivity</strong></td>
<td>Reacts with: Mouse, Rat, Human</td>
</tr>
<tr>
<td><strong>Predicted to work with</strong></td>
<td>Rabbit, Cow</td>
</tr>
<tr>
<td><strong>Immunogen</strong></td>
<td>Synthetic peptide within Human HMGB1 aa 150 to the C-terminus (internal sequence) conjugated to keyhole limpet haemocyanin. The exact sequence is proprietary. Database link: P09429 (Peptide available as ab18650)</td>
</tr>
<tr>
<td><strong>Positive control</strong></td>
<td>Recombinant Human HMGB1 protein (ab73658) can be used as a positive control in WB. This antibody gave a positive signal in the following whole cell lysates: HeLa, Jurkat, A431, HEK 293, NIH 3T3, MEF1, PC12. This antibody gave a positive signal in the following nuclear lysate: HeLa nuclear lysate</td>
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**General notes**

| **Form** | Liquid |
| **Storage instructions** | Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -80°C. Avoid freeze / thaw cycle. |
| **Storage buffer** | pH: 7.40  
Preservative: 0.02% Sodium azide  
Constituent: PBS |
| **Purity** | Immunogen affinity purified |
| **Clonality** | Polyclonal |

Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising agent. If you would like information about the formulation of a specific lot, please contact our scientific support team who will be happy to help.
Isotype

<table>
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<tr>
<th>Applications</th>
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<td><strong>Isotype</strong></td>
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<td><strong>Our Abpromise guarantee</strong> covers the use of <strong>ab18256</strong> in the following tested applications.</td>
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<tr>
<td>The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.</td>
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<tr>
<td><strong>Application</strong></td>
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<tr>
<td>IHC-FoFr</td>
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<td>ICC/IF</td>
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<td>IHC-P</td>
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<td>ChIP</td>
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<td>IHC-Fr</td>
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<td>ICC</td>
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<td>IP</td>
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<td>WB</td>
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**Target**

**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. 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 sensor and/or chaperone for immunogenic nucleic acids implicating the activation of TLR9-mediated immune responses, and mediates autophagy. Acts as danger associated molecular pattern (DAMP) molecule that amplifies immune responses during tissue injury. 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. In the extracellular compartment fully reduced HMGB1 (released by necrosis) acts as a chemokine, disulfide HMGB1 (actively secreted) as a cytokine, and sulfanyl HMGB1 (released from apoptotic cells) promotes immunological tolerance (PubMed:23519706, PubMed:23446148, PubMed:23994764, PubMed:25048472). Has proangiogenic activity (By similarity). May be involved in platelet activation (By similarity). Binds to phosphatidylycerine 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).
Nuclear functions are attributed to fully reduced HGMB1. Associates with chromatin and binds DNA with a preference to non-canonical DNA structures such as single-stranded DNA, DNA-containing cruciforms or bent structures, supercoiled DNA and ZDNA. Can bent DNA and enhance DNA flexibility by looping thus providing a mechanism to promote activities on various gene promoters by enhancing transcription factor binding and/or bringing distant regulatory sequences into close proximity (PubMed:20123072). May have an enhancing role in nucleotide excision repair (NER) (By similarity). However, effects in NER using in vitro systems have been reported conflictingly (PubMed:19446504, PubMed:19360789). May be involved in mismatch repair (MMR) and base excision repair (BER) pathways (PubMed:15014079, PubMed:16143102, PubMed:17803946). May be involved in double strand break repair such as non-homologous end joining (NHEJ) (By similarity). Involved in V(D)J recombination by acting as a cofactor of the RAG complex: acts by stimulating cleavage and RAG protein binding at the 23 bp spacer of conserved recombination signal sequences (RSS) (By similarity). In vitro can displace histone H1 from highly bent DNA (By similarity). Can restructure the canonical nucleosome leading to relaxation of structural constraints for transcription factor-binding (By similarity).

Enhances binding of sterol regulatory element-binding proteins (SREBs) such as SREBF1 to their cognate DNA sequences and increases their transcriptional activities (By similarity). Facilitates binding of TP53 to DNA (PubMed:23063560). Proposed to be involved in mitochondrial quality control and autophagy in a transcription-dependent fashion implicating HSPB1; however, this function has been questioned (By similarity). Can modulate the activity of the telomerase complex and may be involved in telomere maintenance.

In the cytoplasm proposed to dissociate the BECN1:BCL2 complex via competitive interaction with BECN1 leading to autophagy activation (PubMed:20819940). Involved in oxidative stress-mediated autophagy (PubMed:21395369). Can protect BECN1 and ATG5 from calpain-mediated cleavage and thus proposed to control their proautophagic and proapoptotic functions and to regulate the extent and severity of inflammation-associated cellular injury (By similarity). In myeloid cells has a protective role against endotoxemia and bacterial infection by promoting autophagy (By similarity). Involved in endosomal translocation and activation of TLR9 in response to CpG-DNA in macrophages.

In the extracellular compartment (following either active secretion or passive release) involved in regulation of the inflammatory response. Fully reduced HGMB1 (which subsequently gets oxidized after release) in association with CXCL12 mediates the recruitment of inflammatory cells during the initial phase of tissue injury; the CXCL12:HMGB1 complex triggers CXCR4 homodimerization (PubMed:22370717). Induces the migration of monocyte-derived immature dendritic cells and seems to regulate adhesive and migratory functions of neutrophils implicating AGER/RAGE and ITGAM (By similarity). Can bind to various types of DNA and RNA including microbial unmethylated CpG-DNA to enhance the innate immune response to nucleic acids. Proposed to act in promiscuous DNA/RNA sensing which cooperates with subsequent discriminative sensing by specific pattern recognition receptors (By similarity). Promotes extracellular DNA-induced AIM2 inflammasome activation implicating AGER/RAGE (PubMed:24971542). Disulfide HMGB1 binds to transmembrane receptors, such as AGER/RAGE, TLR2, TLR4 and probably TREM1, thus activating their signal transduction pathways. Mediates the release of cytokines/chemokines such as TNF, IL-1, IL-6, IL-8, CCL2, CCL3, CCL4 and CXCL10 (PubMed:12765338, PubMed:18354232, PubMed:19264983, PubMed:20547845, PubMed:24474694). Promotes secretion of interferon-gamma by macrophage-stimulated natural killer (NK) cells in concert with other cytokines like IL-2 or IL-12 (PubMed:15607795). TLR4 is proposed to be the primary receptor promoting macrophage activation and signaling through TLR4 seems to implicate LY96/MD-2 (PubMed:20547845). In bacterial LPS- or LTA-mediated inflammatory responses binds to the endotoxins and transfers them to CD14 for signaling to the respective TLR4:LY96 and TLR2 complexes (PubMed:18354232, PubMed:21660935, PubMed:25660311). Contributes to tumor proliferation by association with ACER/RAGE (By similarity). Can bind to IL1-beta and signals through the IL1R1:IL1RAP receptor complex (PubMed:18250463). Binding to class A
CpG activates cytokine production in plasmacytoid dendritic cells implicating TLR9, MYD88 and AGER/RAGE and can activate autoreactive B cells. Via HMGB1-containing chromatin immune complexes may also promote B cell responses to endogenous TLR9 ligands through a B-cell receptor (BCR)-dependent and ACER/RAGE-independent mechanism (By similarity). Inhibits phagocytosis of apoptotic cells by macrophages; the function is dependent on poly-ADP-ribosylation and involves binding to phosphatidylserine on the cell surface of apoptotic cells (By similarity). In adaptive immunity may be involved in enhancing immunity through activation of effector T cells and suppression of regulatory T (TReg) cells (PubMed:15944249, PubMed:22473704). In contrast, without implicating effector or regulatory T-cells, required for tumor infiltration and activation of T-cells expressing the lymphotoxin LTA:LTB heterotrimer thus promoting tumor malignant progression (By similarity). Also reported to limit proliferation of T-cells (By similarity). Released HMGB1:nucleosome complexes formed during apoptosis can signal through TLR2 to induce cytokine production (PubMed:19064698). Involved in induction of immunological tolerance by apoptotic cells; its pro-inflammatory activities when released by apoptotic cells are neutralized by reactive oxygen species (ROS)-dependent oxidation specifically on Cys-106 (PubMed:18631454). During macrophage activation by activated lymphocyte-derived self apoptotic DNA (ALD-DNA) promotes recruitment of ALD-DNA to endosomes.

Tissue specificity

Sequence similarities
Belongs to the HMGB family.
Contains 2 HMG box DNA-binding domains.

Domain
HMG box 2 mediates proinflammatory cytokine-stimulating activity and binding to TLR4 (PubMed:12765338, PubMed:20547845). However, not involved in mediating immunogenic activity in the context of apoptosis-induced immune tolerance (PubMed:24474694). The acidic C-terminal domain forms a flexible structure which can reversibly interact intramolecularly with the HMG boxes and modulate binding to DNA and other proteins (PubMed:23063560).

Post-translational modifications
Phosphorylated at serine residues. Phosphorylation in both NLS regions is required for cytoplasmic translocation followed by secretion (PubMed:17114460). Acetylated on multiple sites upon stimulation with LPS (PubMed:22801494). Acetylation on lysine residues in the nuclear localization signals (NLS 1 and NLS 2) leads to cytoplasmic localization and subsequent secretion (By similarity). Acetylation on Lys-3 results in preferential binding to DNA ends and impairs DNA bending activity. Reduction/oxidation of cysteine residues Cys-23, Cys-45 and Cys-106 and a possible intramolecular disulfide bond involving Cys-23 and Cys-45 give rise to different redox forms with specific functional activities in various cellular compartments: 1- fully reduced HMGB1 (HMGB1C23hC45hC106h), 2- disulfide HMGB1 (HMGB1C23-C45-C106h) and 3- sulfonyl HMGB1 (HMGB1C23soC45soC106so). Poly-ADP-ribosylated by PARP1 when secreted following stimulation with LPS. In vitro cleavage by CASP1 is liberating a HMG box 1-containing peptide which may mediate immunogenic activity; the peptide antagonizes apoptosis-induced immune tolerance (PubMed:24474694). Can be proteolytically cleaved by a thrombin:thrombomodulin complex; reduces binding to heparin and proinflammatory activities.

Cellular localization
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).

**Images**

**All lanes**: Anti-HMGB1 antibody - ChIP Grade (ab18256) at 1 µg/ml

**Lane 1**: Wild-type HAP1 whole cell lysate

**Lane 2**: HMGB1 knockout HAP1 whole cell lysate

Lysates/proteins at 20 µg per lane.

**Predicted band size**: 25 kDa

**Lanes 1 - 4**: Merged signal (red and green). Green - ab18256 observed at 29 kDa. Red - loading control, ab9484, observed at 37 kDa.

ab18256 was shown to recognize HMGB1 in wild-type HAP1 cells as signal was lost at the expected MW in HMGB1 knockout cells. Additional cross-reactive bands were observed in the wild-type and knockout cells. Wild-type and HMGB1 knockout samples were subjected to SDS-PAGE. Ab18256 and ab9484 (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1 µg/ml and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed ab216773 and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed ab216776 secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.
Localisation and expression of HMGB1

HMGB1 nuclear expression (brown staining) was seen in all pancreatic compartments, including stromal immune infiltrate: top panels show PanIN-1 (left) and -2 (right) (magnification ×50, insert and second panel x100); bottom panels show PanIN-3 (left) and PDAC (right) (magnification ×100 and ×50, respectively).

PDCA = pancreatic adenocarcinoma. PanIN = precursor lesions.

ab18256 is used at 1/1000 dilution.

(After Figure 5 B of Crnogorac-Jurcevic et al)

ab18256 stained in Hela cells. Cells were fixed with 4% paraformaldehyde (10min) at room temperature and incubated with PBS containing 10% goat serum, 0.3 M glycine, 1% BSA and 0.1% triton for 1 hour at room temperature to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody ab18256 at 1 µg/ml and ab7291 (Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control) at 1/1000 dilution overnight at +4°C. The secondary antibodies were Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) preadsorbed (ab150120) (pseudo-colored red) and Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed (ab150081) secondary antibody (colored green) used at 1 ug/ml for 1 hour at room temperature. DAPI was used to stain the cell nuclei (colored blue) at a concentration of 1.43 µM for 1 hour at room temperature.

ab18256 used in Direct ELISA in NIH 3T3 murine fibroblasts. Primary antibody used at a 1/1000 dilution for 16 hours at 4°C. The secondary antibody is an AP-conjugated Goat anti-rabbit used at a 1/1000 dilution. A blocking step was performed using 5% BSA for 1 hour.
Immunochemistry (Frozen sections) - Anti-HMGB1 antibody - ChIP Grade (ab18256)
This image is courtesy of an anonymous Abreview.

Western blot - Anti-HMGB1 antibody - ChIP Grade (ab18256)

**All lanes**: Anti-HMGB1 antibody - ChIP Grade (ab18256) at 1 µg/ml

**Lane 1**: NIH 3T3 whole cell lysate ([ab7179](#))
**Lane 2**: MEF1 (Mouse embryonic fibroblast cell line) Whole Cell Lysate
**Lane 3**: PC12 (Rat adrenal pheochromocytoma cell line) Whole Cell Lysate

Lysates/proteins at 10 µg per lane.

**Secondary**
**All lanes**: IRDye 680 Conjugated Goat Anti-Rabbit IgG (H+L) at 1/10000 dilution

Performed under reducing conditions.

**Predicted band size**: 25 kDa
**Observed band size**: 29 kDa

why is the actual band size different from the predicted?

**Additional bands at**: 59 kDa. We are unsure as to the identity of these extra bands.

ab18256 staining HMGB1 in Human stomach tissue sections by IHC-Fr (Immunohistochemistry - Frozen sections). Tissue samples were fixed with acetone and blocked with 5% serum for 1 hour at 25°C. Samples were incubated with primary antibody 1/500 in blocking buffer for 1 hour at 25°C. An undiluted HRP-conjugated Goat polyclonal to rabbit IgG was used as secondary antibody.
All lanes: Anti-HMGB1 antibody - ChIP Grade (ab18256) at 1 µg/ml

Lane 1: HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate

Lane 2: Jurkat whole cell lysate (ab7899)

Lane 3: A431 whole cell lysate (ab7909)

Lane 4: HEK293 whole cell lysate (ab7902)

Lysates/proteins at 10 µg per lane.

Secondary

All lanes: IRDye 680 Conjugated Goat Anti-Rabbit IgG (H+L) at 1/10000 dilution

Performed under reducing conditions.

Predicted band size: 25 kDa

Observed band size: 29 kDa why is the actual band size different from the predicted?

Image courtesy of Human Protein Atlas

Paraffin embedded sections of human liver were incubated with ab18256 (1/1000 dilution) for 30 minutes at room temperature.

Heat induced antigen retrieval was performed in citrate buffer pH 6.

ab18256 was tested in a tissue microarray (TMA) containing a wide range of normal and cancer tissues as well as a cell microarray consisting of a range of commonly used, well characterised human cell lines. Further images can be found www.proteinatlas.org
ab18256 staining HMGB1 in mouse liver tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Tissue was fixed with formaldehyde and blocked with 15% serum for 60 minutes at 20°C; antigen retrieval was by heat mediation. Samples were incubated with primary antibody (1/1000 in TBS) for 18 hours at 20°C. An Alexa Fluor® 647-conjugated goat anti-rabbit IgG polyclonal (1/500) was used as the secondary antibody.

All lanes: Anti-HMGB1 antibody - ChIP Grade (ab18256) at 1/1000 dilution

Lane 1: Rat brain whole tissue lysate - infused with asf for 1 week
Lane 2: Rat brain whole tissue lysate - infused with LPS for 1 week
Lane 3: Rat brain whole tissue lysate - infused with acsf for 8 weeks
Lane 4: Rat brain whole tissue lysate - infused with LPS for 8 weeks
Lane 5: Rat brain whole tissue lysate - infused with LPS for 4 weeks
Lane 6: Rat brain whole tissue lysate - infused with LPS for 4 weeks, after 2 weeks of LPS infusion were treated with neramexane for the next 2 weeks
Lane 7: Rat brain whole tissue lysate - infused with LPS for 4 weeks, after 2 weeks of LPS infusion were treated with memantine for the next 2 weeks.

Lysates/proteins at 40 µg per lane.

Secondary
All lanes: Biotinylated Goat anti-rabbit IgG

Developed using the ECL technique.

Performed under reducing conditions.
Predicted band size: 25 kDa

Exposure time: 30 seconds

Anti-HMGB1 antibody - ChIP Grade (ab18256) at 1 µg/ml + Recombinant Human HMGB1 protein (ab56525) at 0.01 µg

Secondary
Goat Anti-Rabbit IgG H&L (HRP) preadsorbed (ab97080) at 1/5000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 25 kDa

Exposure time: 2 minutes

ab18256 staining HMGB1 in murine kidney tissue by Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections).
Tissue was fixed in formaldehyde and a heat mediated antigen retrieval step was performed using citrate EDTA buffer pH 6.2. Samples were then blocked, then incubated with ab18256 at a 1/1000 dilution for 1 hour. The secondary used was a goat anti-rabbit IgG conjugated to HRP at a 1/1000 dilution.

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