

Anti-HMGB1 antibody [EPR3507]

Anti-HMGB1 antibody [EPR3507] (ab79823) is a rabbit monoclonal antibody detecting HMGB1 in **Western Blot, Flow Cytometry (Intra), IHC-P, ICC/IF**. Suitable for **Human, Rat**.

- KO validated for confirmed specificity
- Biophysical QC for unrivalled batch-batch consistency
- Over 270 publications
- Trusted since 2009

Recombinant

RabMAb

KO Validated

20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EPR3507
Purification technique	Affinity purification Protein A
Concentration	0.171 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Not recommended

Species	Rat, Human, Mouse
Dilution info	-
Notes	-

WB

Tested	
Species	Rat
Dilution info	1/10000 - 1/50000
Notes	-
Species	Human
Dilution info	1/10000 - 1/50000
Notes	-
Species	Mouse
Dilution info	-
Notes	-

ICC/IF

Tested	
Species	Human
Dilution info	1/250
Notes	-

Expected

Species	Rat, Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

Flow Cyt (Intra)

Tested	
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Species	Human
Dilution info	1/30
Notes	ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Expected

Species	Rat, Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

IHC-P

Tested

Species	Human
Dilution info	1/350 - 1/400
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Species	Mouse
Dilution info	1/350
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Species	Rat
Dilution info	1/350
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Target data

[See full target information HMGB1](#) [↗](#)

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
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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 proangiogenic 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). 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:19360789, PubMed:19446504). 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 (SREBPs) 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 (By similarity). 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 (By similarity). 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 AGER/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 AGER/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 (By similarity). (Microbial infection) Critical for entry of human coronaviruses SARS-CoV and SARS-CoV-2, as well as human coronavirus NL63/HCoV-NL63 (PubMed:33147444). Regulates the expression of the pro-viral genes ACE2 and CTSL through chromatin modulation (PubMed:33147444). Required for SARS-CoV-2 ORF3A-induced reticulophagy which induces endoplasmic reticulum stress and inflammatory responses and facilitates viral infection (PubMed:35239449). (Microbial infection) Associates with the influenza A viral protein NP in the nucleus of infected cells, promoting viral growth and enhancing the activity of the viral polymerase. (Microbial infection) Promotes Epstein-Barr

virus (EBV) latent-to-lytic switch by sustaining the expression of the viral transcription factor BZLF1 that acts as a molecular switch to induce the transition from the latent to the lytic or productive phase of the virus cycle. Mechanistically, participates in EBV reactivation through the NLRP3 inflammasome. (Microbial infection) Facilitates dengue virus propagation via interaction with the untranslated regions of viral genome. In turn, this interaction with viral RNA may regulate secondary structure of dengue RNA thus facilitating its recognition by the replication complex.

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Stable for 12 months at -20°C

Notes

Anti-HMGB1 antibody [EPR3507] (ab79823) was developed by Abcam using patented rabbit monoclonal antibody technology and is validated for use in Flow Cyt (Intra), ICC/IF, IHC-P and WB.

Anti-HMGB1 antibody [EPR3507] (ab79823) was first used in a scientific publication in 2011 and has been cited over 275 times in peer reviewed journals. It's performance in Western Blot in human, mouse and rat samples is trusted by the scientific community.

Abcam's high quality manufacturing and validation processes ensure Anti-HMGB1 antibody [EPR3507] (ab79823) has high sensitivity and specificity alongside high lot-to-lot consistency and reproducibility.

The specificity of Anti-HMGB1 antibody [EPR3507] (ab79823) has been confirmed by Western Blot testing in HMGB1 knockout HeLa cells (ab255395).

Anti-HMGB1 antibody [EPR3507] (ab79823) has 7 independent reviews from customers.

Anti-HMGB1 antibody [EPR3507] (ab79823) specifically detects HMGB1 (UniProt ID: P09429; Molecular weight: 25kDa) and is sold in 100 µL and 1 mL selling sizes.

Conjugation-ready, carrier free format available for antibody clone EPR3507 - ab216986.

Antibody clone EPR3507 is also available pre-conjugated to a variety of labels for your convenience - Alexa Fluor®

488, Alexa Fluor® 647, HRP, Alexa Fluor® 594, Alexa Fluor® 405, Alexa Fluor® 555, APC, PE (ab195010, ab195011, ab195012, ab206894, ab206895, ab206896, ab225041, ab225042).

HMGB1 promotes inflammation and tissue damage in diseases like sepsis, cancer and autoimmune disorders by acting as a damage-associated molecular pattern (DAMP) and activating immune responses.

Patented technology

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?

This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free batch production

For more information, read more on recombinant antibodies.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

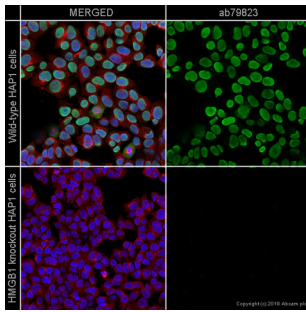
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
[Terms & Conditions](#).

18 product images

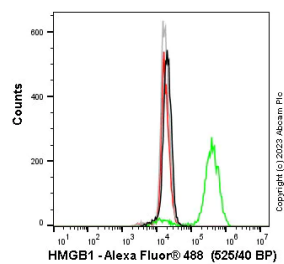


Immunocytochemistry/ Immunofluorescence - Anti-HMGB1 antibody [EPR3507] (ab79823)

HMGB1 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-HMGB1 antibody

ab79823 staining HMGB1 in wild-type HAP1 cells (top panel) and HMGB1 knockout HAP1 cells (bottom panel). The cells were fixed with 4% formaldehyde for 10 minutes, permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1 hour. The cells were then incubated with ab79823 at 1/250 dilution and ab195889 at 1/250 dilution (shown in pseudo colour red) overnight at +4°C, followed by a further incubation at room temperature for 1 hour with Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed (ab150081) secondary antibody at 2 µg/ml (shown in green). Nuclear DNA was labelled in blue with DAPI.

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Flow Cytometry (Intracellular) - Anti-HMGB1 antibody [EPR3507] (ab79823)

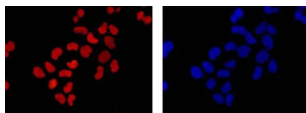
HMGB1 Flow Cytometry (Intracellular) staining using rabbit Anti-HMGB1 antibody

Flow cytometry overlay histogram showing wild-type Hap1 (green line) and HMGB1 knockout Hap1 stained with ab79823 (red line). The cells were fixed with 4% formaldehyde (10 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (ab79823) (1x 10⁶ in 100µl at 0.008 µg/ml (1/260000)) for 30min at 22°C.

The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 for 30min at 22°C

Isotype control antibody Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control was used at the same concentration and conditions as the primary antibody (wild-type Hap1 - black line, HMGB1 knockout Hap1 - grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity).

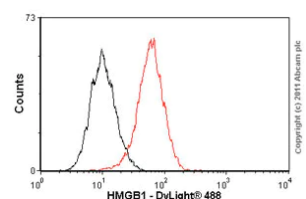
Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.



Immunocytochemistry/ Immunofluorescence - Anti-HMGB1 antibody [EPR3507] (ab79823)

HMGB1 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-HMGB1 antibody

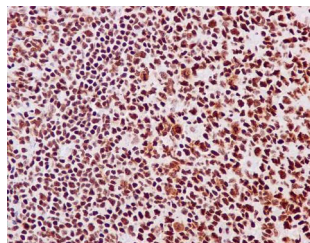
Immunocytochemistry/Immunofluorescence analysis of HeLa cells labelling HMGB1 (red) with unpurified ab79823 at 1/350. Cells were fixed with 4% paraformaldehyde. An Alexa Fluor® 555-conjugated goat anti-rabbit IgG (1/200) was used as the secondary antibody. Counterstained with DAPI (blue).



Flow Cytometry (Intracellular) - Anti-HMGB1 antibody [EPR3507] (ab79823)

HMGB1 Flow Cytometry (Intracellular) staining using rabbit Anti-HMGB1 antibody

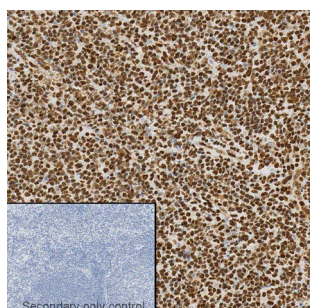
Overlay histogram showing HeLa (Human epithelial cell line from cervix adenocarcinoma) cells stained with unpurified ab79823 (red line). The cells were fixed with methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab79823, 1/20 dilution) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-rabbit IgG (H+L) ([ab96899](#)) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit monoclonal IgG (0.5µg/1x10⁶ cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a decreased signal in HeLa cells fixed with 4% paraformaldehyde/permeabilized with 0.1% PBS-Tween 20 used under the same conditions.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HMGB1 antibody [EPR3507] (ab79823)

HMGB1 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-HMGB1 antibody

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human tonsil tissue labeling HMGB1 with unpurified ab79823 at 1/350. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. A prediluted HRP-polymer conjugated anti-rabbit IgG was used as the secondary antibody. Counterstained with Hematoxylin.



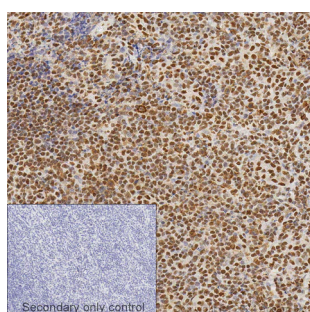
Immunohistochemistry - Anti-HMGB1 antibody [EPR3507] (ab79823)

HMGB1 Immunohistochemistry staining of Human tonsil using rabbit Anti-HMGB1 antibody

Immunohistochemical analysis of formalin fixed paraffin embedded human tonsil labelling HMGB1 with ab79823 at a concentration of 0.1µ/ml. The immunostaining was performed on a Ventana DISCOVERY ULTRA (Roche Tissue Diagnostics) instrument with a OptiView DAB IHC Detection Kit. Heat mediated antigen retrieval was performed with DISCOVERY cell conditioning solution (CC1) 100°C, pH8.5 for 32mins.

ab79823 anti-HMGB1 antibody [EPR3507] was incubated for 16mins at 37°C. Sections were counterstained with Hematoxylin II. Image inset shows absence of staining in secondary antibody only control.

Customers are encouraged to optimise antigen retrieval conditions, antibody concentration, incubation times and temperature for best results in their own IHC assay workflow (automated and manual)



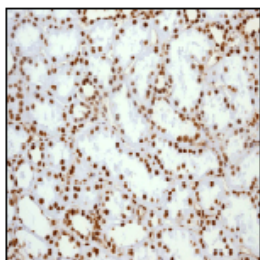
Immunohistochemistry - Anti-HMGB1 antibody [EPR3507] (ab79823)

HMGB1 Immunohistochemistry staining of Human tonsil using rabbit Anti-HMGB1 antibody

Immunohistochemical analysis of formalin fixed paraffin embedded human tonsil labelling HMGB1 with ab79823 at a concentration of 0.1µ/ml. The immunostaining was performed on a Leica Biosystems BOND® RX instrument with a Bond™ Polymer Refine Detection kit. Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution 2) for 20mins.

ab79823 anti-HMGB1 antibody [EPR3507] was incubated for 15mins at room temperature. Sections were counterstained with Hematoxylin. Image inset shows absence of staining in secondary antibody only control.

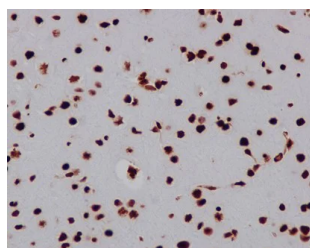
Customers are encouraged to optimise antigen retrieval conditions, antibody concentration, incubation times and temperature for best results in their own IHC assay workflow (automated and manual)



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HMGB1 antibody [EPR3507] (ab79823)

HMGB1 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-HMGB1 antibody

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human kidney tissue labeling HMGB1 with unpurified ab79823 at 1/250 dilution. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HMGB1 antibody [EPR3507] (ab79823)

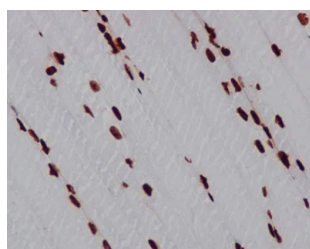
HMGB1 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Rat brain using rabbit Anti-HMGB1 antibody

Immunohistochemical analysis of paraffin-embedded rat brain tissue labeling HMGB1 with ab79823 at 1/350 dilution.

Positive staining on rat brain.

The section was incubated with ab79823 incubated at +4 °C overnight followed by a Goat Anti-Rabbit IgG H&L (HRP polymer) ready to use secondary. Counterstained with Hematoxylin.

Heat mediated antigen retrieval using [ab93684](#) (Tris/EDTA buffer, pH 9.0)



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HMGB1 antibody [EPR3507] (ab79823)

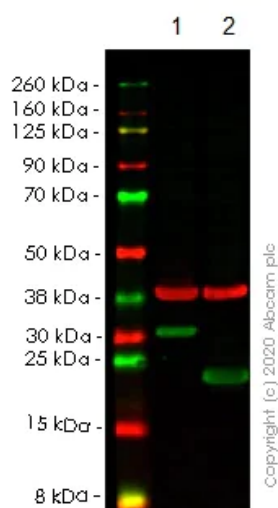
HMGB1 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Mouse muscle using rabbit Anti-HMGB1 antibody

Immunohistochemical analysis of paraffin-embedded mouse muscle tissue labeling HMGB1 with ab79823 at 1/350 dilution.

Positive staining on mouse muscle.

The section was incubated with ab79823 incubated at +4 °C overnight followed by a Goat Anti-Rabbit IgG H&L (HRP polymer) ready to use secondary. Counterstained with Hematoxylin.

Heat mediated antigen retrieval using [ab93684](#) (Tris/EDTA buffer, pH 9.0)



Western blot - Anti-HMGB1 antibody [EPR3507] (ab79823)

HMGB1 Western blot staining using rabbit Anti-HMGB1 antibody

Lanes 1- 2: Merged signal (red and green). Green - ab79823 observed at 30 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (ab8245) observed at 37 kDa.

ab79823 was shown to react with HMGB1 in wild-type HeLa cells in western blot. Loss of signal at the expected size was observed when CRISPR/Cas9 edited cell line ab255395 (CRISPR/Cas9 edited cell lysate ab263782) was used. The band observed in lane 2 below 25kDa may represent truncated forms and cleaved fragments. Wild-type HeLa and HMGB1 CRISPR/Cas9 edited HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab79823 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) overnight at 4°C at a 1 in 10000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye®800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye®680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

All lanes:

Western blot - Anti-HMGB1 antibody [EPR3507] (ab79823) at 1/10000 dilution

Lane 1:

Wild-type HeLa cell lysate at 20 µg

Lane 2:

HMGB1 CRISPR/Cas9 edited HeLa cell lysate at 20 µg

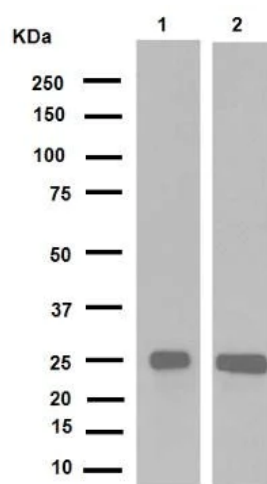
Lane 2:

Western blot - Human HMGB1 knockout HeLa cell line (ab255395)

Performed under reducing conditions.

Predicted band size: 24 kDa

Observed band size: 30 kDa



Western blot - Anti-HMGB1 antibody [EPR3507] (ab79823)

Blocking/Dilution buffer and concentration: 5% NFDM/TBST.

All lanes:

Western blot - Anti-HMGB1 antibody [EPR3507] (ab79823) at 1/10000 dilution

Lane 1:

SK-BR-3 (Human mammary gland adenocarcinoma cell line) cell lysate at 10 µg

Lane 2:

HeLa (Human epithelial cell line from cervix adenocarcinoma) cell lysate at 10 µg

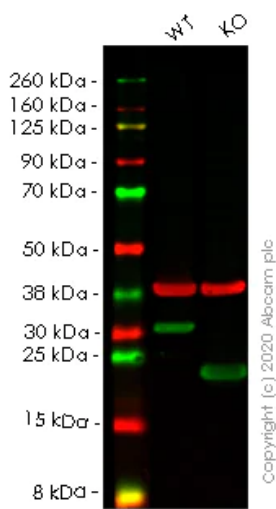
Secondary

All lanes:

Peroxidase-conjugated goat anti-rabbit IgG (H+L) at 1/1000 dilution

Predicted band size: 24 kDa

Observed band size: 25 kDa



Western blot - Anti-HMGB1 antibody [EPR3507] (ab79823)

HMGB1 Western blot staining using rabbit Anti-HMGB1 antibody

Lanes 1- 2: Merged signal (red and green). Green - ab79823 observed at 30 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (ab8245) observed at 37 kDa.

ab79823 was shown to react with HMGB1 in wild-type HeLa cells in western blot. Loss of signal at the expected size was observed when knockout cell line ab255395 (knockout cell lysate ab263782) was used. The band observed in lane 2 below 25kDa may represent truncated forms and cleaved fragments. Wild-type HeLa and HMGB1 knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab79823 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) overnight at 4°C at a 1 in 10000 Dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye®800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye®680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

All lanes:

Western blot - Anti-HMGB1 antibody [EPR3507] (ab79823) at 1/10000 dilution

Lane 1:

Wild-type HeLa cell lysate at 20 µg

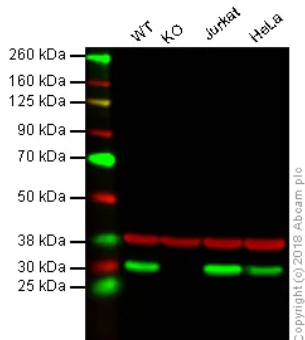
Lane 2:

HMGB1 knockout HeLa cell lysate at 20 µg

Performed under reducing conditions.

Predicted band size: 24 kDa

Observed band size: 30 kDa



Western blot - Anti-HMGB1 antibody [EPR3507] (ab79823)

Lanes 1 - 4: Merged signal (red and green). Green - ab79823 observed at 30 kDa. Red - loading control, ab9484, observed at 37 kDa.

ab79823 was shown to specifically react with HMGB1 in wild-type HAP1 cells as signal was lost in HMGB1 knockout cells. Wild-type and HMGB1 knockout samples were subjected to SDS-PAGE. ab79823 and ab9484 (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1/10000 dilution 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.

All lanes:

Western blot - Anti-HMGB1 antibody [EPR3507] (ab79823) at 1/10000 dilution

Lane 1:

Wild-type HAP1 whole cell lysate at 20 µg

Lane 2:

HMGB1 knockout HAP1 whole cell lysate at 20 µg

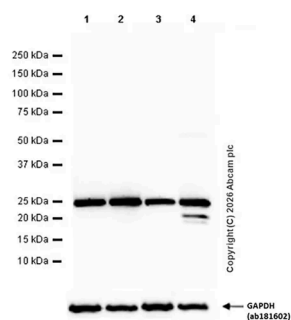
Lane 3:

Jurkat whole cell lysate at 20 µg

Lane 4:

HeLa whole cell lysate at 20 µg

Predicted band size: 24 kDa



Western blot - Anti-HMGB1 antibody [EPR3507] (ab79823)

HMGB1 Western blot staining using rabbit Anti-HMGB1 antibody

Blocking and diluting buffer and concentration: 5% NFDm/TBST.

In Western blot, Anti-GAPDH antibody [EPR16891] - Loading Control ([ab181602](#)).

All lanes:

Western blot - Anti-HMGB1 antibody [EPR3507] (ab79823) at 1/1000 dilution

Lane 1:

Mouse brain tissue lysate at 20 µg

Lane 2:

Mouse kidney tissue lysate at 20 µg

Lane 3:

Rat brain tissue lysate at 20 µg

Lane 4:

Rat kidney tissue lysate at 20 µg

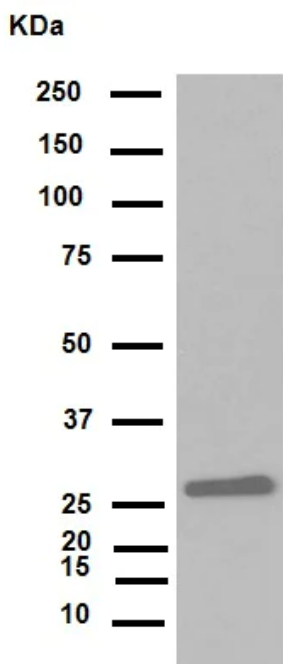
Secondary

All lanes:

Western blot - Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 1/20000 dilution

Observed band size: 25 kDa, 36 kDa

Exposure time: 3s



Western blot - Anti-HMGB1 antibody [EPR3507] (ab79823)

HMGB1 Western blot staining of Rat brain tissue lysate using rabbit Anti-HMGB1 antibody

Blocking/Dilution buffer and concentration: 5% NFDm/TBST.

All lanes:

Western blot - Anti-HMGB1 antibody [EPR3507] (ab79823) at 1/10000 dilution

All lanes:

Rat brain tissue lysate at 10 µg

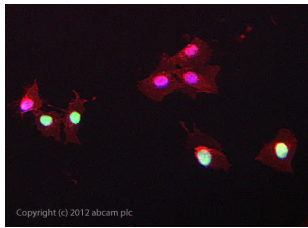
Secondary

All lanes:

Peroxidase-conjugated goat anti-rabbit IgG (H+L) at 1/1000 dilution

Predicted band size: 24 kDa

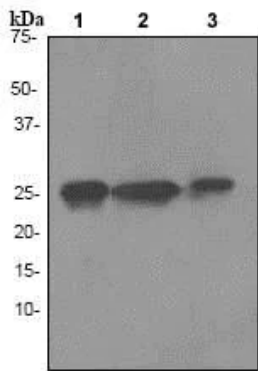
Observed band size: 25 kDa



Immunocytochemistry/ Immunofluorescence - Anti-HMGB1 antibody [EPR3507] (ab79823)

HMGB1 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-HMGB1 antibody

ICC/IF image of unpurified ab79823 stained DU 145 (Human prostate carcinoma cell line) cells. The cells were 4% formaldehyde fixed (10 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilize the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody ab79823 at 1/1000 dilution overnight at +4°C. The secondary antibody (green) was DyLight® 488 goat anti- rabbit ([ab96899](#)) IgG (H+L) used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.



Western blot - Anti-HMGB1 antibody [EPR3507] (ab79823)

All lanes:

Western blot - Anti-HMGB1 antibody [EPR3507] (ab79823) at 1/50000 dilution

Lane 1:

SK-BR-3 cell lysate at 10 µg

Lane 2:

HeLa cell lysate at 10 µg

Lane 3:

HepG2 cell lysate at 10 µg

Secondary

All lanes:

Goat anti-rabbit HRP conjugate at 1/2000 dilution

Predicted band size: 24 kDa

Observed band size: 25 kDa

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.