HMGB1 Polyclonal Antibody

Catalog Number PA1-16926

Product data sheet

Details
Size 100 µL
Host/Isotype Rabbit / IgG
Class Polyclonal
Type Antibody
Immunogen Synthetic peptide between to amino acids 100-200 of the human HMGB1 protein.
Conjugate Unconjugated
Form Liquid
Concentration 1 mg/mL
Purification Antigen affinity chromatography
Storage buffer tris glycine
Contains 0.02% sodium azide
Storage Conditions 4°C, do not freeze

Species Reactivity
Species reactivity
Published species
Bovine, Dog, Human, Mouse, Sheep, Rabbit, Rat
Dog, Rat, Human, Not Applicable

Tested Applications
Dilution *
ChiP assay (ChIP) 5 µg/mL
ELISA (ELISA) Assay-Dependent
Flow Cytometry (Flow) 1:100
Immunohistochemistry (Paraffin) (IHC (P)) 1:100-1:250
Western Blot (WB) 0.5-1 µg/mL
Immunocytochemistry (ICC/IF) 0.05 µg/mL

Published Applications
Published species
Immunohistochemistry (ICC/IF) See 1 publications below
Immunohistochemistry (Paraffin) (IHC (P)) See 1 publications below
Miscellaneous PubMed (Misc) See 1 publications below

Background/Target Information
HMGB1 (High-mobility group box-1) protein was originally described as a nuclear non-histone DNA binding chromosomal protein. However, recent studies indicate that damaged, necrotic cells liberate HMGB1 into the extracellular milieu where it functions as a proinflammatory cytokine. Mouse HMGB1 is expressed as a 215 amino acid single chain polypeptide containing three domains: two tandem-linked positively charged DNA-binding domains (HMGB box A, aa 9-79; and box B, aa 89-162), and a negatively charged 30 aa C-terminal acidic tail region. Residues 28 - 44 and 180 - 185 contain a nuclear localization signal (NLS). The cytokine activity of HMGB1 is contained in the B box, while the A box is associated with the helix-loop-helix domain of transcription factors. HMGB1 acts both as an inflammatory mediator that promotes monocyte migration and cytokine secretion, as well as a mediator of T cell-dendritic cell interaction. HMGB1 is secreted and acts to transduce cellular signals through its high affinity receptor, RAGE and possibly, TLR2 and TLR4. HMGB1 is highly conserved and ubiquitous in the nuclei and cytoplasm of nearly all cell types, is a necessary and sufficient mediator of inflammation during sterile and infection-associated responses. HMGB1 also act as DNA nuclear binding protein that has recently been shown to be an early trigger of sterile inflammation in animal models of trauma-hemorrhage via the activation of the Toll-like receptor 4 and the receptor for the advanced glycation endproducts (RAGE). Moreover, HMGB1 is reported that the level of HMGB1 is elevated during sterile tissue injury, infection, lethal endotoxemia or sepsis, collagen-induced arthritis, and ischemia-reperfusion induced tissue injury.

Product Images For HMGB1 Polyclonal Antibody

HMGB1 Antibody (PA1-16926)
Antibody specificity was demonstrated by CRISPR-Cas9 mediated knockout of target protein. A loss of signal was observed for target protein in HMGB1 KO cell line compared to control cell line using Anti-HMGB1 Polyclonal Antibody (Product # PA1-16926). [KO]

HMGB1 Antibody (PA1-16926) in ICC/IF
Immunofluorescence analysis of HMGB1 was performed using 70% confluent log phase SH-SY5Y cells. The cells were fixed with 4% paraformaldehyde for 10 minutes, permeabilized with 0.1% Triton X-100 for 15 minutes, and blocked with 2% BSA for 45 minutes at room temperature. The cells were labeled with HMGB1 Polyclonal Antibody (Product # PA1-16926) at 0.05 µg/mL in 0.1% BSA, incubated at 4 degree celsius overnight and then labeled with Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor Plus 488 (Product # A32790), (1:2000), for 45 minutes at room temperature (Panel a: Blue). Nuclei (Panel b: Green) were stained with ProLong™ Diamond Antifade Mountant with DAPI (Product # P36962). F-actin (Panel c: Red) was stained with Rhodamine Phalloidin (Product # R415, 1:300). Panel d represents the merged image showing Nuclear localization. Panel e represents control cells with no primary antibody to assess background. The images were captured at 60X magnification.

HMGB1 Antibody (PA1-16926) in IHC (P)
Immunohistochemical analysis of HMGB1 in mouse liver. Samples were incubated in HMGB1 polyclonal antibody (Product # PA1-16926).

HMGB1 Antibody (PA1-16926)
Antibody specificity was demonstrated by siRNA mediated knockdown of target protein. MCF7 cells were transfected with HMGB1 siRNA and decrease in signal intensity was observed in Western Blot application using Anti-HMGB1 Polyclonal Antibody (Product # PA1-16926). [KD]
HMGB1 Antibody (PA1-16926) in WB
Knockout of HMGB1 was achieved by CRISPR-Cas9 genome editing using LentiArray™ Lentiviral sgRNA (Product # A32042, Assay ID CRISPR784665_LV) and LentiArray Cas9 Lentivirus (Product # A32064). Western blot analysis of HMGB1 was performed by loading 30 µg of HeLa Wild Type (Lane 1), HeLa Cas9 (Lane 2) and HeLa HMGB1 KO (Lane 3) whole cell extracts. The samples were electrophoresed using NuPAGE™ Novex™ 4-12% Bis-Tris Protein Gel (Product # NP0322BOX). Resolved proteins were then transferred onto a nitrocellulose membrane (Product # IB23001) by iBlot® 2 Dry Blotting System (Product # IB21001). The blot was probed with Anti-HMGB1 Polyclonal Antibody (Product # PA1-16926, 1:1,000 dilution) and Goat anti-Rabbit IgG (H+L) Superclonal™ Recombinant Secondary Antibody, HRP (Product # A27036, 1:5,000 dilution) using the iBright FL 1000 (Product # A32752). Chemiluminescent detection was performed using Novex® ECL Chemiluminescent Substrate Reagent Kit (Product # WP20005). Loss of signal upon CRISPR mediated knockout (KO) using the LentiArray™ CRISPR product line confirms that antibody is specific to HMGB1. An uncharacterized band was observed in all the samples at ~50 kDa.

HMGB1 Antibody (PA1-16926) in WB
Western blot was performed using Anti-HMGB1 Polyclonal Antibody (Product # PA1-16926) and a ~30kDa band corresponding to HMGB1 was observed across cell lines and tissues tested. Whole cell extracts (30 µg lysate) of RAW 264.7 (Lane 1), MCF7 (Lane 2), SH-SY5Y (Lane 3), THP-1 (Lane 4), PC-12 (Lane 5), HEK-293 (Lane 6), A-431 (Lane 7), Hep G2 (Lane 8), HeLa (Lane 9), Mouse Liver (Lane 10), Mouse Ovary (Lane 11) were electrophoresed using NuPAGE™ 4-12% Bis-Tris Protein Gel (Product # NP0321BOX). Resolved proteins were then transferred onto a Nitrocellulose membrane (Product # LC2001) by iBlot® 2 Dry Blotting System (Product # IB21001). The blot was probed with the primary antibody (1:1000 dilution) and detected by chemiluminescence with Goat anti-Rabbit IgG (H+L) Superclonal™ Recombinant Secondary Antibody, HRP (Product # A27036, 1:4000) using the iBright FL 1000 (Product # A32752). Chemiluminescent detection was performed using Novex® ECL Chemiluminescent Substrate Reagent Kit (Product # WP20005).

HMGB1 Antibody (PA1-16926) in WB
Knockdown of HMGB1 was achieved by transfecting MCF7 with HMGB1 specific siRNAs (Silencer® select Product # s20255, s20254). Western blot analysis (Fig. a) was performed using Whole cell extracts from the HMGB1 knockdown cells (lane 3), non-targeting scrambled siRNA transfected cells (lane 2) and untransfected cells (lane 1). The blot was probed with HMGB1 Polyclonal Antibody (Product # PA1-16926, 1 µg/mL) and Goat anti-Rabbit IgG (H+L) Superclonal™ Recombinant Secondary Antibody, HRP (Product # A27036, 1:4000). Densitometric analysis of this western blot is shown in histogram (Fig. b). Decrease in signal upon siRNA mediated knock down confirms that antibody is specific to HMGB1.

HMGB1 Antibody (PA1-16926) in WB
Western blot analysis of HMGB1 in SHSY-5Y, MCF7, Neuro2A and HeLa. Samples were incubated in HMGB1 polyclonal antibody (Product # PA1-16926) using a dilution of 2 µg/mL followed by an anti-rabbit HRP secondary antibody. Separated on a 12% gel by SDS-PAGE, transferred to PVDF membrane and blocked in 5% non-fat milk in TBST. Detection: chemiluminescence.
HMGB1 Antibody (PA1-16926) in WB
Western blot analysis of HMGB1 in 0.05 mg/mL Jurkat lysate. Samples were incubated in HMGB1 polyclonal antibody (Product # PA1-16926). This experiment was performed under reducing conditions using the 12-230 kDa separation system.

HMGB1 Antibody (PA1-16926) in Flow
Flow cytometry of HMGB1 in HeLa and a matched isotype control. Samples were incubated in HMGB1 polyclonal antibody (Product # PA1-16926) using a dilution of 1 µg/mL for 30 minutes at room temperature followed by a Rabbit IgG (H+L) Cross-Adsorbed secondary antibody. Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin.

HMGB1 Antibody (PA1-16926) in Flow
Flow cytometry of HMGB1 in RH-30 cells. Samples were incubated in HMGB1 polyclonal antibody (Product # PA1-16926) using a dilution of 10 µg/mL for 30 minutes at room temperature. Antibody (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin.

HMGB1 Antibody (PA1-16926) in ChIP
Chromatin Immunoprecipitation (ChIP) assay of endogenous HMGB1 protein using HMGB1 Antibody: ChIP was performed using HMGB1 Polyclonal Antibody (Product # PA1-16926, 5 µg) on sheared chromatin from Hep G2 cells using the MAGnify ChIP System kit (Product # 49-2024). Normal Rabbit IgG was used as a negative IP control. The purified DNA was analyzed by qPCR using primers binding to MIA-PR (promoter), c myc promoter, TNF alpha (active) and SAT alpha and SAT2 satellite repeats (Inactive). Data is presented as fold enrichment of the antibody signal versus the negative control IgG using the comparative CT method.
### PubMed References For HMGB1 Polyclonal Antibody

#### 1 Immunocytochemistry References

<table>
<thead>
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<th>Species / Dilution</th>
<th>Summary</th>
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<tbody>
<tr>
<td>Human / 1:50</td>
<td>PA1-16926 was used in Immunocytochemistry-immunofluorescence to explore the potential of NB-PDT to stimulate the immune system.</td>
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#### 1 Immunohistochemistry (Paraffin) References

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<th>Species / Dilution</th>
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<td>Human / 4 µg/ml</td>
<td>PA116926 was used in immunohistochemistry - paraffin section to analyze immunogenic biomarkers and immune cells infiltration and prognosis in metastasis.</td>
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#### 1 Miscellaneous PubMed References

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<td>Human / Not Cited</td>
<td>PA1-16926 was used in immunohistochemistry - paraffin section to examine the immune responses in patients with esophageal carcinomas treated by neo-adjuvant radiochemotherapy</td>
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