Human/Mouse HMGB1/HMG-1 Antibody

Antigen Affinity-purified Polyclonal Chicken IgY
Catalog Number: AF1690

DESCRIPTION

Species Reactivity	Human/Mouse
Specificity	Detects human and mouse HMGB1/HMG-1 in direct ELISAs and Western blots. In direct ELISAs, approximately 15% cross-reactivity with recombinant human (rh) HMGB3 is observed, and less than 1% cross-reactivity with rhHMGA1, rhHMGA2, and rhHMGA1b is observed.
Source	Polyclonal Chicken IgY
Purification	Antigen Affinity-purified
Immunogen	Mouse myeloma cell line NS0-derived recombinant human HMGB1/HMG-1 Met1-Glu215 Accession # P09429.3
Formulation	Lyophilized from a 0.2 μm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 μm filtered solution in PBS.

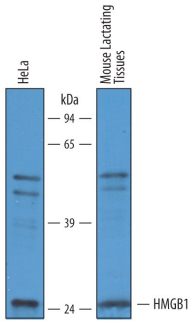
APPLICATIONS

Please Note: *Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.*

	Recommended Concentration	Sample
Western Blot	1 µg/mL	See Below
Knockout Validated	HMGB1/HMG-1 is specifically detected in HEK293T human embryonic kidney parental cell line but is not detectable in HMGB1/HMG-1 knockout HEK293T cell line.	

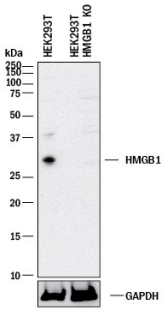
DATA

Western Blot



Detection of Human and Mouse HMGB1/HMG-1 by Western Blot. Western blot shows lysates of HeLa human cervical epithelial carcinoma cell line and mouse lactating tissues. PVDF membrane was probed with 1 µg/mL of Chicken Anti-Human/Mouse HMGB1/HMG-1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1690) followed by HRP-conjugated Anti-Chicken IgY Secondary Antibody. A specific band was detected for HMGB1/HMG-1 at approximately 25 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 8.

Knockout Validated



Western Blot Shows Human HMGB1/HMG-1 Specificity by Using Knockout Cell Line. Western blot shows lysates of HEK293T human embryonic kidney parental cell line and HMGB1/HMG-1 knockout HEK293T cell line (KO). PVDF membrane was probed with 1 µg/mL of Chicken Anti-Human/Mouse HMGB1/HMG-1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1690) followed by HRP-conjugated Anti-Chicken IgY Secondary Antibody. A specific band was detected for HMGB1/HMG-1 at approximately 30 kDa (as indicated) in the parental HEK293T cell line, but is not detectable in knockout HEK293T cell line. GAPDH (Catalog # AF5718) is shown as a loading control. This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** ● 12 months from date of receipt, -20 to -70 °C as supplied. ● 1 month, 2 to 8 °C under sterile conditions after reconstitution. ● 6 months, -20 to -70 °C under sterile conditions after reconstitution.

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Global bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL +1 612 379 2956
USA TEL 800 343 7475 Canada TEL 855 668 8722 China TEL +86 (21) 52380373
Europe | Middle East | Africa TEL +44 (0)1235 529449

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BACKGROUND

Human High-mobility group box 1 protein (HMGB1), previously known as HMG-1 or amphoterin, is a member of the high mobility group box family of non-histone chromosomal proteins (1-3). Human HMGB1 is expressed as a 25-30 kDa, 215 amino acid (aa) single chain polypeptide containing three domains: two N-terminal globular, 70 aa positively charged DNA-binding domains (HMG boxes A and B), and a negatively charged 30 aa C-terminal region that contains only Asp and Glu (4, 5). Residues 27-43 and 178-184 contain a NLS. Posttranslational modifications of the molecule have been reported, with acetylation occurring on as many as 17 lysine residues (6). HMGB1 is expressed at high levels in almost all cells (2, 4). It was originally discovered as a nuclear protein that could bend DNA. Such bending stabilizes nucleosome formation and regulates the expression of select genes upon recruitment by DNA binding proteins (1, 7, 8). It is now known that HMGB1 can also act extracellularly, both as an inflammatory mediator that promotes monocyte migration and cytokine secretion, and as a mediator of T cell-dendritic cell interaction (1, 4, 7, 9, 10). The cytokine activity of HBMG1 is restricted to the HMG B box, (3) while the A box is associated with the helix-loop-helix domain of transcription factors (11). HMBG1 is released in response to cell death and as a secretion product. Although HMBG-1 does not possess a classic signal sequence, it appears to be secreted as an acetylated form via secretory endolysosome exocytosis (6, 12). Once secreted, HMGB1 transduces cellular signals through its high affinity receptor, RAGE and, possibly, TLR2 and TLR4 (1, 3, 4). Human HMGB1 is 100% aa identical to canine HMGB1 and 99% aa identical to mouse, rat, bovine and porcine HMGB1, respectively.

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