

DESCRIPTION

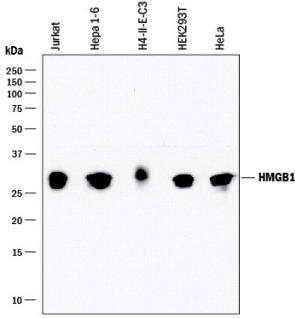
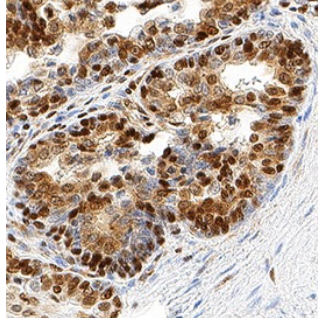
Species Reactivity	Human/Mouse/Rat
Specificity	Detects human, mouse, and rat HMGB1/HMG-1 in Western blot.
Source	Monoclonal Rat IgG _{2B} Clone # 951420
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	Human HMGB1/HMG-1 synthetic peptide Accession # P09429
Formulation	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	0.1 µg/mL	See Below
Immunohistochemistry	5-25 µg/mL	See Below

DATA

<p>Western Blot</p>  <p>Detection of Human, Mouse, and Rat HMGB1/HMG-1 by Western Blot. Western blot shows lysates of Jurkat human acute T cell leukemia cell line, Hepa 1-6 mouse hepatoma cell line, H4-II-E-C3 rat hepatoma cell line, HEK293 human embryonic kidney cell line, and HeLa human cervical epithelial carcinoma cell line. PVDF membrane was probed with 0.1 µg/mL of Rat Anti-Human HMGB1/HMG-1 Monoclonal Antibody (Catalog # MAB16901) followed by HRP-conjugated Anti-Rat IgG Secondary Antibody (Catalog # HAF005). A specific band was detected for HMGB1/HMG-1 at approximately 28 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.</p>	<p>Immunohistochemistry</p>  <p>HMGB1/HMG-1 in Human Prostate Cancer Tissue. HMGB1/HMG-1 was detected in immersion fixed paraffin-embedded sections of human prostate cancer tissue using Rat Anti-Human/Mouse/Rat HMGB1/HMG-1 Monoclonal Antibody (Catalog # MAB16901) at 5 µg/mL overnight at 4 °C. Tissue was stained using the Anti-Rat HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS017) and counterstained with hematoxylin (blue). Specific staining was localized to nuclei and cytoplasm. View our protocol for Chromogenic IHC Staining of Paraffin-embedded Tissue Sections.</p>
--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------	-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.5 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. <ul style="list-style-type: none"> ● 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.

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 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.

References:

1. Lotze, M.T. and K.J. Tracey (2005) *Nat. Rev. Immunol.* **5**:331.
2. Yang, H. *et al.* (2005) *J. Leukoc. Biol.* **78**:1.
3. Dumitriu, I.E. *et al.* (2005) *Trends Immunol.* **26**:381.
4. Degryse, B. and M. de Virgilio (2003) *FEBS Lett.* **553**:11.
5. Wen, L. *et al.* (1989) *Nucleic Acids Res.* **17**:1197.
6. Bonaldi, T. *et al.* (2003) *EMBO J.* **22**:5551.
7. Muller, S. *et al.* (2001) *EMBO J.* **20**:4337.
8. Bustin, M. (1999) *Mol. Cell. Biol.* **19**:5237.
9. Wang, H. *et al.* (1999) *Science.* **285**:248.
10. Dimitriu, I.E. *et al.* (2005) *J. Immunol.* **174**:7506.
11. Najima, Y. *et al.* (2005) *J. Biol. Chem.* **280**:27523.
12. Gardella, S. *et al.* (2002) *EMBO Rep.* **3**:995.