

DESCRIPTION

Species Reactivity	Human/Mouse
Specificity	Detects human HMGB1 in direct ELISAs. Detects human and mouse HMGB1 in Western blots.
Source	Monoclonal Mouse IgG _{2B} Clone # 1002147
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	Synthetic peptide containing human HMGB1 Thr85-Gly166 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.

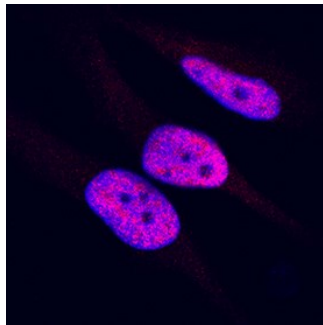
APPLICATIONS

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
Immunocytochemistry	8-25 µg/mL	See Below

DATA

Immunocytochemistry



HMGB1/HMG-1 in HeLa Human Cell Line.

HMGB1/HMG-1 was detected in immersion fixed HeLa human cervical epithelial carcinoma cell line using Mouse Anti-Human/Mouse HMGB1/HMG-1 Monoclonal Antibody (Catalog # MAB16902) at 8 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Mouse IgG Secondary Antibody (red; Catalog # NL007) and counterstained with DAPI (blue). Specific staining was localized to nuclei. View our protocol for [Fluorescent ICC Staining of Cells on Coverslips](#).

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). HMGB1 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. Dumitriu, 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.