Human HMGB1/HMG-1 Antibody

Monoclonal Mouse IgG_{2B} Clone # 115603

Catalog Number: MAB1690

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human HMGB1/HMG-1 in direct ELISAs and Western blots.
Source	Monoclonal Mouse IgG_{2B} Clone # 115603
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	*E. coli*-derived recombinant human HMGB1/HMG-1 Gly2-Glu215 (predicted) 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 as a 0.2 μm filtered solution in PBS.

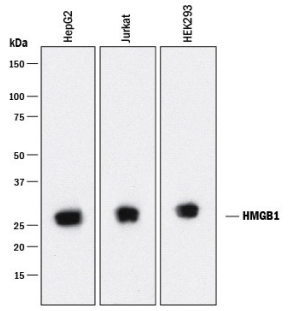
APPLICATIONS

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	0.05 µg/mL	See Below
Chromatin Immunoprecipitation (ChIP)	5 µg/5 x 10^6 cells	See Below
Immunocytochemistry	8-25 µg/mL	See Below
Intracellular Staining by Flow Cytometry	2.5 µg/10^6 cells	See Below
CyTOF-ready	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	

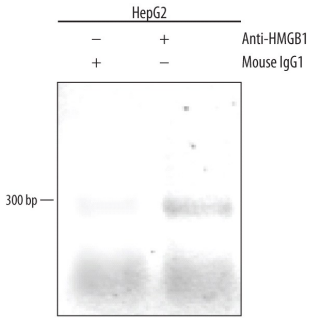
DATA

Western Blot



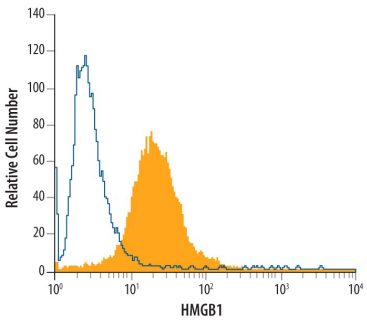
Detection of Human HMGB1/HMG-1 by Western Blot. Western blot shows lysates of HepG2 human hepatocellular carcinoma cell line, Jurkat human acute T cell leukemia cell line, and HEK293 human embryonic kidney cell line. PVDF membrane was probed with 0.05 µg/mL of Mouse Anti-Human HMGB1/HMG-1 Monoclonal Antibody (Catalog # MAB1690) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF018). A specific band was detected for HMGB1/HMG-1 at approximately 26 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Chromatin Immunoprecipitation (ChIP)



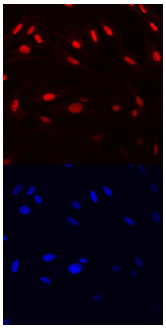
Detection of HMGB1/HMG-1-regulated Genes by Chromatin Immunoprecipitation. HepG2 human hepatocellular carcinoma cell line was fixed using formaldehyde, resuspended in lysis buffer, and sonicated to shear chromatin. HMGB1/HMG-1/DNA complexes were immunoprecipitated using 5 µg Mouse Anti-Human HMGB1/HMG-1 PE-conjugated Monoclonal Antibody (Catalog # MAB1690) or control antibody (Catalog # MAB004) for 15 minutes in an ultrasonic bath, followed by Biotinylated Anti-Mouse IgG Secondary Antibody (Catalog # BAF007). Immunocomplexes were captured using 50 µL of MagCellect Streptavidin Ferrofluid (Catalog # MAG999) and DNA was purified using chelating resin solution. The *AFP* promoter was detected by standard PCR.

Intracellular Staining by Flow Cytometry



Detection of HMGB1 in HCT-116 Human Cell Line by Flow Cytometry. HCT-116 human colorectal carcinoma cell line was stained with Mouse Anti-Human HMGB1/HMG-1 Monoclonal Antibody (Catalog # MAB1690, filled histogram) or isotype control antibody (Catalog # MAB0041, open histogram), followed by Phycoerythrin-conjugated Anti-Mouse IgG $F(ab')_2$ Secondary Antibody (Catalog # F0102B). To facilitate intracellular staining, cells were fixed with Flow Cytometry Fixation Buffer (Catalog # FC004) and permeabilized with Flow Cytometry Permeabilization/Wash Buffer I (Catalog # FC005). View our protocol for Staining Intracellular Molecules.

Immunocytochemistry



HMGB1/HMG-1 in CCD-18Co Human Cell Line. HMGB1/HMG-1 was detected in immersion fixed CCD-18Co human colonic fibroblast cell line using Mouse Anti-Human HMGB1/HMG-1 Monoclonal Antibody (Catalog # MAB1690) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Mouse IgG Secondary Antibody (red, upper panel; Catalog # NL007) and counterstained with DAPI (blue, lower panel). Specific staining was localized to nuclei. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

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Human HMGB1/HMG-1 Antibody

Monoclonal Mouse IgG_{2B} Clone # 115603

Catalog Number: MAB1690

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.** ● 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:

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