

#### DESCRIPTION

<b>Species Reactivity</b>	Human
<b>Specificity</b>	Detects human HMGB1/HMG-1 in direct ELISAs and Western blots.
<b>Source</b>	Monoclonal Mouse IgG <sub>2B</sub> Clone # 115603
<b>Purification</b>	Protein A or G purified from hybridoma culture supernatant
<b>Immunogen</b>	<i>E. coli</i> -derived recombinant human HMGB1/HMG-1 Gly2-Glu215 (predicted) Accession # P09429
<b>Conjugate</b>	Alexa Fluor 750 Excitation Wavelength: 749 nm Emission Wavelength: 775 nm
<b>Formulation</b>	Supplied 0.2 mg/mL in a saline solution containing BSA and Sodium Azide. See Certificate of Analysis for details.  *Contains <0.1% Sodium Azide, which is not hazardous at this concentration according to GHS classifications. Refer to the Safety Data Sheet (SDS) for additional information and handling instructions.

#### 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
<b>Intracellular Staining by Flow Cytometry</b>	0.25-1 µg/10 <sup>6</sup> cells	HCT-116 human colorectal carcinoma cell line fixed with Flow Cytometry Fixation Buffer (Catalog # FC004) and permeabilized with Flow Cytometry Permeabilization/Wash Buffer I (Catalog # FC005)

#### PREPARATION AND STORAGE

<b>Shipping</b>	The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.
<b>Stability &amp; Storage</b>	<b>Protect from light. Do not freeze.</b> <ul style="list-style-type: none"> <li>12 months from date of receipt, 2 to 8 °C as supplied.</li> </ul>

#### 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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