HMGB1 Mouse Monoclonal Antibody

MCA-1F3

Ordering Information
Web *www.encorbio.com*
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Phone *352-372-7022*
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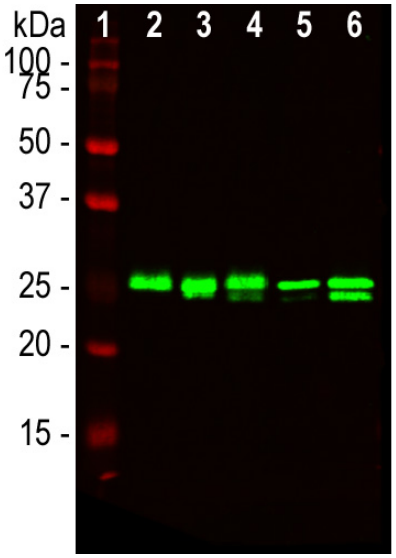
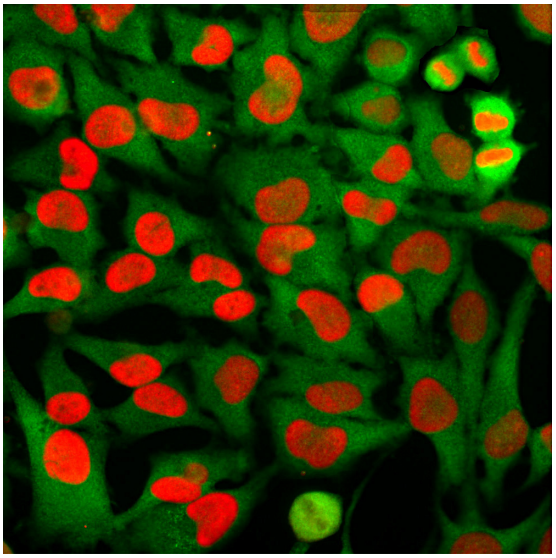
HGNC Name: *HMGB1*
UniProt: *P09429*
RRID: *AB_2572333*
Immunogen: *Human full length recombinant human HMGB1 protein expressed in and purified from E. coli.*
Format: *Purified antibody at 1mg/mL in 50% PBS, 50% glycerol plus 5mM NaN_3*
Storage: *Stable at 4°C for one year, for longer term store at -20°C*
Recommended dilutions:
WB: 1:1,000-1:2,000. ICC/IF and IHC: 1:1,000

Applications	Host	Isotype	Molecular Wt.	Species Cross-Reactivity
WB, IF/ICC, IHC	Mouse	IgG2b	25kDa	Hu, Rt, Ms



Western blot analysis of lysates from different cell lines probed with mouse mAb to high mobility group protein B1, (HMGB1), MCA-1F3, dilution 1:2,000: [1] protein standard, [2] NIH-3T3, [3] C6, [4] HEK293, [5] HeLa, and [6] SH-SY5Y. The 25kDa band revealed by MCA-1F3 antibody corresponds to the HMGB1 protein.



Immunofluorescent analysis of of HeLa cells stained with mouse mAb to HMGB1, MCA-1F3, dilution 1:2,000 in red and costained with rabbit pAb to GAPDH, RPCA-GAPDH, dilution 1:2,000 in green. MCA-1F3 antibody stains the chromatin binding protein HMGB1, which is localized in the nuclei. In contrast the RPCA-GAPDH antibody produces strong cytoplasmic staining of cells.

Background:

High-mobility group proteins were named originally since they are abundant, relatively low molecular weight and exhibit "high mobility", in other words, they run quickly on SDS-PAGE gels. High-mobility group proteins box 1 (HMGB1) is one of these. The “box” in the name refers to the so-called high mobility group box, a compact domain involved in DNA binding and protein-protein interactions. The HMGB1 molecule has two of these HMG domains (1). The protein is also called amphoterin, this name being derived from the presence of two highly charged regions in the molecule, a relatively neutrally charged N-terminus and a very negatively charged C-terminus. The molecule is very unusually charged throughout, the human sequence consisting of 16.7% Glutamic acid, 9.3% Aspartic acid, 20% Lysine and 9.3% Arginine. HMGB1 can bind toll like receptor 2, 4 and 9 (TLR2, TLR4 and TLR9) and the receptor for advanced glycation end products (RAGE), (3,4). TLRs are components of the innate immune system, first recognized as a family of receptors which recognize “pathogen associated molecular pattern" molecules or (PAMPs). These are common components of bacteria and when TLRs bind these, a strong inflammatory response is activated. More recently it has been recognized that TLRs can also be activated by "damage associated molecular pattern" molecules or (DAMPs), substances released from damaged and diseased cells which also bind to TLR family receptors and also activate inflammation. HMGB1 is such a DAMP, binding to TLR4, and much evidence suggests that HMGB1 is a strong activator of inflammation. Interestingly, HMGB1 is released by necrotic cells but not by apoptotic cells (4).

The MCA-1F3 was made against the full length recombinant HMGB1 molecule expressed in and purified from *E. coli*. The antibody works well on western blots of cell and tissue extracts and for IHC imaging of human, rat and mouse samples. The HMGB1 protein is normally located in the nucleus where it associates with chromatin and DNA, so the antibody is also useful nuclear marker.

References:

1. Lotze MT, Tracey KJ. High-mobility group box 1 protein (HMGB1): nuclear weapon in the immune arsenal. Nat. Rev. Immunol.5:331-42 (2005).
2. Park J, et al. Involvement of TLR 2 and TLR 4 in Cellular Activation by High Mobility Group Box 1 protein (HMGB1). J. Biol. Chem. 27;279:7370 (2004).
3. Tian J, et al. Toll-like receptor 9–dependent activation by DNA-containing immune complexes is mediated by HMGB1 and RAGE. Nat. Immunol. :487-96 (2007).
4. Scaffidi P, Misteli T, Bianchi ME. Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. Nature 418:191-5 (2002).

Abbreviation Key:

mAb—Monoclonal Antibody pAb—Polyclonal Antibody WB—Western Blot IF—Immunofluorescence ICC—Immunocytochemistry IHC—Immunohistochemistry E—ELISA Hu—Human Mo—Monkey Do—Dog Rt—Rat Ms—Mouse Co—Cow Pi—Pig Ho—Horse Ch—Chicken Dr—D. rerio Dm—D. melanogaster Sm—S. mutans Ce—C. elegans Sc—S. cerevisiae Sa—S. aureus Ec—E. coli.