Product datasheet

Anti-HMGB1 Antibody (Clone#5H3)

Catalog Number: *M00066-2*

BOSTER
antibody and ELISA experts
BOSTER BIOLOGICAL TECHNOLOGY
Special NO.1, International Enterprise Center,
2nd Guanshan Road, Wuhan, China
Web: www.boster.com.cn ***Phone:*** +86 027-67845390 ***Fax:*** +86 027-67845390 ***Email:*** boster@boster.com.cn

Basic Information

Product Name	Anti-HMGB1 Antibody (Clone#5H3)
Gene Name	HMGB1
Source	Mouse
Isotype	IgG2b
Species Reactivity	human, monkey, mouse, rat
Tested Application	WB, IHC, FCM
Contents	500 ug/ml antibody with PBS， 0.02% NaN3 , 1 mg BSA and 50% glycerol.
Immunogen	A synthetic peptide corresponding to a sequence at the C-terminus of human HMGB1 (124-154aa DVAKKLGEMWNNTAADDKQPYEKKAAKLKEK), identical to the related mouse and rat sequences.
concentration	500 ug/ml
Purification	protein G purified.
Observed MW	25KD
Dilution Ratios	Western blot(WB): 1:500-2000 Immunohistochemistry in paraffin section (IHC): 1:50-400 Flow cytometry (FCM): 1-3 μg/1x10^6 cells (Boiling the paraffin sections in 10mM citrate buffer,pH6.0,or PH8.0 EDTA repair liquid for 20 mins is required for the staining of formalin/paraffin sections.) Optimal working dilutions must be determined by end user.

Storage

12 months from date of receipt，-20℃ as supplied. 6 months 2 to 8℃ after reconstitution. Avoid repeated freezing and thawing.

Selected Validation Data

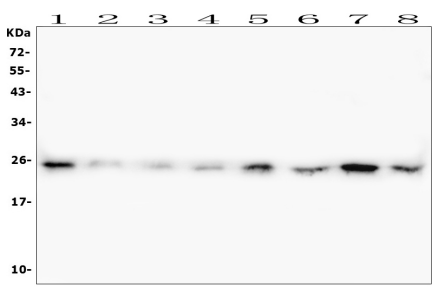

Figure 1. Western blot analysis of HMGB1 using anti-HMGB1 antibody (M00066-2).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.

Lane 1: human HepG2 whole cell lysates

BOSTER
antibody and ELISA experts
BOSTER BIOLOGICAL TECHNOLOGY
Special NO.1, International Enterprise Center,
2nd Guanshan Road, Wuhan, China
Web: www.boster.com.cn ***Phone:*** +86 027-67845390 ***Fax:*** +86 027-67845390 ***Email:*** boster@boster.com.cn

Lane 2: human CCRF-CEM whole cell lysates

Lane 3: monkey COS-7 whole cell lysates

Lane 4: human SW620 whole cell lysates

Lane 5: human THP-1 whole cell lysates

Lane 6: rat PC-12 whole cell lysates

Lane 7: rat RH35 whole cell lysates

Lane 8: mouse NIH/3T3 whole cell lysates
After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA for 50-90 minutes. Blocked the membrane with 5% Non-fat Milk/ TBS for 1.5 hour at RT. The membrane was incubated with mouse anti-HMGB1 antigen affinity purified monoclonal antibody (Catalog # M00066-2) at 0.5 µg/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-mouse IgG-HRP secondary antibody at a dilution of 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1001) with Tanon 5200 system. A specific band was detected for HMGB1 at approximately 25KD. The expected band size for HMGB1 is at 25KD.

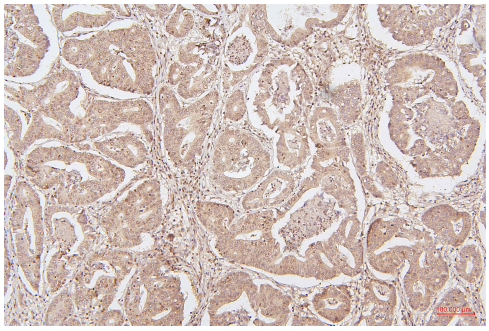

Figure 2. IHC analysis of HMGB1 using anti-HMGB1 antibody (M00066-2).
HMGB1 was detected in paraffin-embedded section of human intestinal cancer tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1µg/ml mouse anti-HMGB1 Antibody (M00066-2) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

BOSTER
antibody and ELISA experts
BOSTER BIOLOGICAL TECHNOLOGY
Special NO.1, International Enterprise Center,
2nd Guanshan Road, Wuhan, China
Web: www.boster.com.cn ***Phone:*** +86 027-67845390 ***Fax:*** +86 027-67845390 ***Email:*** boster@boster.com.cn

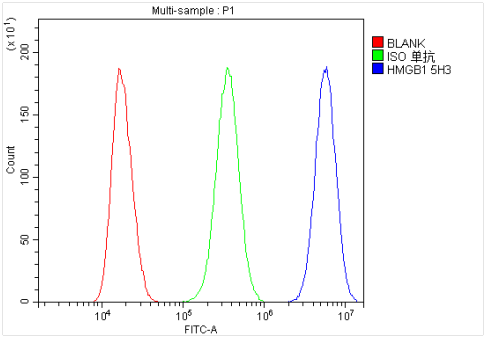

Figure 14. Flow Cytometry analysis of SiHa cells using anti-HMGB1 antibody (M00066-2).
Overlay histogram showing SiHa cells stained with M00066-2 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-HMGB1 Antibody (M00066-2,1µg/1x10^6 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10µg/1x10^6 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1µg/1x10^6) used under the same conditions. Unlabelled sample (Red line) was also used as a control.