Anti-HMGB1 Antibody [SA39-03]

ET1601-2

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, FC
Molecular Wt:	Predicted band size: 25 kDa
Clone number:	SA39-03

Description: High mobility group box 1 protein, also known as high-mobility group protein 1 (HMG-1) and amphoterin, is a protein that in humans is encoded by the HMGB1 gene. HMG-1 belongs to the high mobility group and contains a HMG-box domain. Like the histones, HMGB1 is among the most important chromatin proteins. In the nucleus HMGB1 interacts with nucleosomes, transcription factors, and histones. This nuclear protein organizes the DNA and regulates transcription. After binding, HMGB1 bends DNA, which facilitates the binding of other proteins. HMGB1 supports transcription of many genes in interactions with many transcription factors. It also interacts with nucleosomes to loosen packed DNA and remodel the chromatin. Contact with core histones changes the structure of nucleosomes. The presence of HMGB1 in the nucleus depends on posttranslational modifications. When the protein is not acetylated, it stays in the nucleus, but hyperacetylation on lysine residues causes it to translocate into the cytosol. HMGB1 has been shown to play an important role in helping the RAG endonuclease form a paired complex during V(D)J recombination.

Immunogen: Synthetic peptide within Human HMGB1 aa 151-200 / 215.

Positive control: HepG2 cell lysate, HeLa cell lysate, HCT 116 cell lysate, A549 cell lysate, Jurkat cell lysate, C2C12 cell lysate, C6 cell lysate, HeLa, mouse thymus tissue lysate, rat spleen tissue lysate, MCF-7, human tonsil tissue, human kidney tissue, mouse brain tissue, PC-12, mouse hippocampus tissue.

Subcellular location: Cytoplasm, Nucleus, Cell membrane, Secreted, Chromosome

Database links: SwissProt: P09429 Human | P63158 Mouse | P63159 Rat

Recommended Dilutions:

WB	1:20,000-1:50,000
IF-Cell	1:500
IF-Tissue	1:100
IHC-P	1:5,000-1:20,000
FC	1:50-1:1,000

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

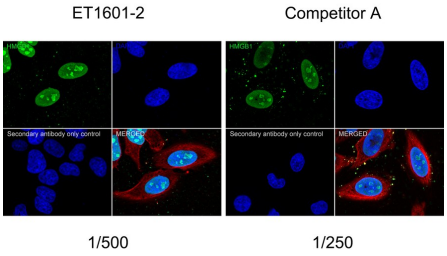

Fig1: Immunocytochemistry analysis of HeLa cells labeling HMGB1 with Rabbit anti-HMGB1 antibody (ET1601-2) at 1/500 dilution and competitor's antibody at 1/250 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-HMGB1 antibody (ET1601-2) at 1/500 dilution and competitor's antibody at 1/250 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

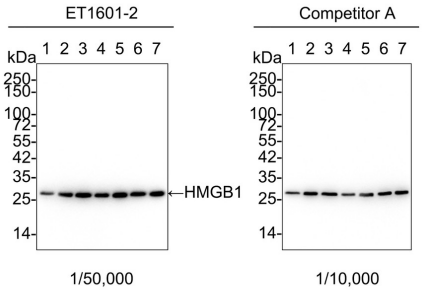

Fig2: Western blot analysis of HMGB1 on different lysates with Rabbit anti-HMGB1 antibody (ET1601-2) at 1/50,000 dilution and competitor's antibody at 1/10,000 dilution.

Lane 1: HepG2 cell lysate
Lane 2: HeLa cell lysate
Lane 3: HCT 116 cell lysate
Lane 4: A549 cell lysate
Lane 5: Jurkat cell lysate
Lane 6: C2C12 cell lysate
Lane 7: C6 cell lysate

Lysates/proteins at 15 µg/Lane.

Predicted band size: 25 kDa
Observed band size: 25 kDa

Exposure time: 21 seconds;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1601-2) at 1/50,000 dilution and competitor's antibody at 1/10,000 dilution were used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

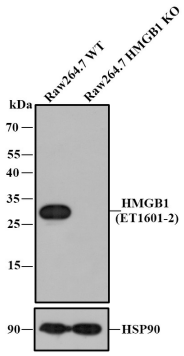

Fig3: All lanes: Western blot analysis of HMGB1 with anti-HMGB1 antibody [SA39-03] (ET1601-2) at 1:500 dilution.

Lane 1: Wild-type Raw264.7 whole cell lysate.
Lane 2: HMGB1 knockout Raw264.7 whole cell lysate.

ET1601-2 was shown to specifically react with HMGB1 in wild-type Raw264.7 cells. No band was observed when HMGB1 knockout samples were tested. Wild-type and HMGB1 knockout samples were subjected to SDS-PAGE. Proteins were transferred to a PVDF membrane and blocked with 5% NFDM in TBST for 1 hour at room temperature. The primary Anti-HMGB1 antibody (ET1601-2, 1/500) and Anti-HSP90 antibody (ET1605-56, 1/10,000) were used in 5% BSA at room temperature for 2 hours. Goat Anti-Rabbit IgG H&L (HRP) Secondary Antibody (HA1001) at 1:200,000 dilution was used for 1 hour at room temperature.

Cell lysate was provided by Ubigene Biosciences (Ubigene Biosciences Co., Ltd., Guangzhou, China).

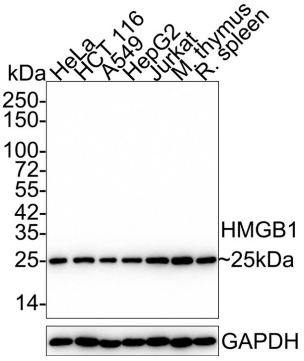

Fig4: Western blot analysis of HMGB1 on different lysates with Rabbit anti-HMGB1 antibody (ET1601-2) at 1/20,000 dilution.

Lane 1: HeLa cell lysate (20 µg/Lane)
Lane 2: HCT 116 cell lysate (20 µg/Lane)
Lane 3: A549 cell lysate (20 µg/Lane)
Lane 4: HepG2 cell lysate (20 µg/Lane)
Lane 5: Jurkat cell lysate (20 µg/Lane)
Lane 6: Mouse thymus tissue lysate (20 µg/Lane)
Lane 7: Rat spleen tissue lysate (30 µg/Lane)

Predicted band size: 25 kDa
Observed band size: 25 kDa

Exposure time: 3 minutes 10 seconds;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1601-2) at 1/20,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

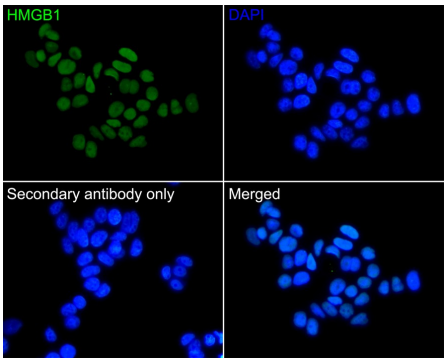

Fig5: Immunocytochemistry analysis of MCF-7 cells labeling HMGB1 with Rabbit anti-HMGB1 antibody (ET1601-2) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 ℃, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-HMGB1 antibody (ET1601-2) at 1/100 dilution in 2% negative goat serum overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

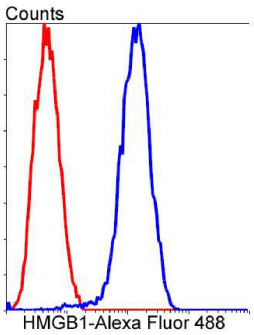

Fig6: Flow cytometric analysis of HMGB1 was done on PC-12 cells. The cells were fixed, permeabilized and stained with the primary antibody (ET1601-2, 1/50) (blue). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor 488-conjugated Goat anti-Rabbit IgG Secondary antibody at 1/1000 dilution for 30 minutes.Unlabelled sample was used as a control (cells without incubation with primary antibody; red).

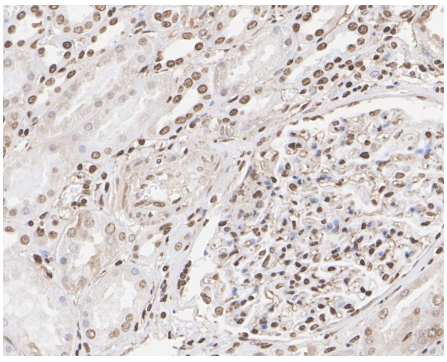

Fig7: Immunohistochemical analysis of paraffin-embedded human kidney tissue with Rabbit anti-HMGB1 antibody (ET1601-2) at 1/20,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1601-2) at 1/20,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

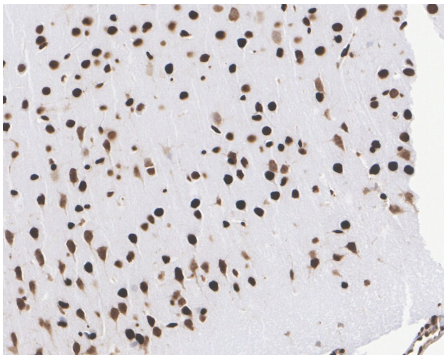

Fig8: Immunohistochemical analysis of paraffin-embedded mouse brain tissue with Rabbit anti-HMGB1 antibody (ET1601-2) at 1/20,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1601-2) at 1/20,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

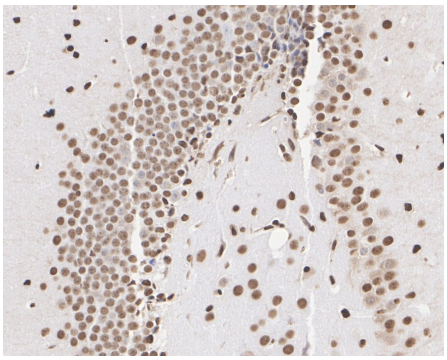

Fig9: Immunohistochemical analysis of paraffin-embedded mouse hippocampus tissue with Rabbit anti-HMGB1 antibody (ET1601-2) at 1/20,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1601-2) at 1/20,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

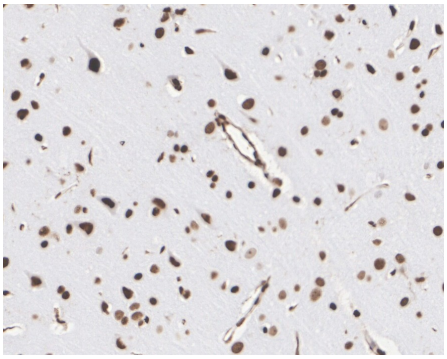

Fig10: Immunohistochemical analysis of paraffin-embedded rat brain tissue with Rabbit anti-HMGB1 antibody (ET1601-2) at 1/20,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1601-2) at 1/20,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

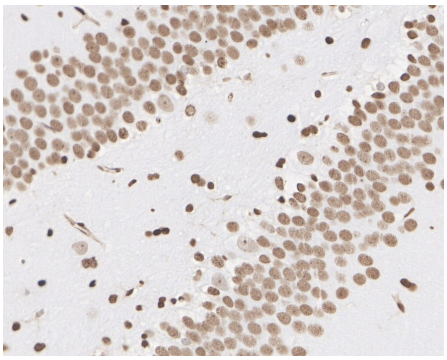

Fig11: Immunohistochemical analysis of paraffin-embedded rat hippocampus tissue with Rabbit anti-HMGB1 antibody (ET1601-2) at 1/20,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1601-2) at 1/20,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

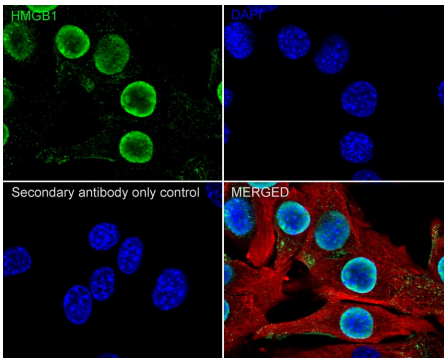

Fig12: "Immunocytochemistry analysis of C2C12 cells labeling HMGB1 with Rabbit anti-HMGB1 antibody (ET1601-2) at 1/500 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-HMGB1 antibody (ET1601-2) at 1/500 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

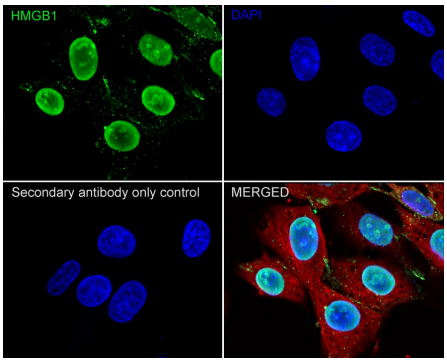

Fig13: Immunocytochemistry analysis of C6 cells labeling HMGB1 with Rabbit anti-HMGB1 antibody (ET1601-2) at 1/500 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-HMGB1 antibody (ET1601-2) at 1/500 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

Background References

1. "Novel role of PKR in inflammasome activation and HMGB1 release." Lu B., Nakamura T., Inouye K., Li J., Tang Y., Lundbaeck P., Valdes-Ferrer S.I., Olofsson P.S., Kalb T., Roth J., Zou Y., Erlandsson-Harris H., Yang H., Ting J.P., Wang H., Andersson U., Antoine D.J., Chavan S.S., Hotamisligil G.S., Tracey K.J. Nature 488:670-674(2012).
2. "The genetic variation of the human HMGB1 gene." Kornblit B., Munthe-Fog L., Petersen S., Madsen H., Vindeloev L., Garred P. Tissue Antigens 70:151-156(2007).