For Research Use Only.
Not for use in diagnostic procedures.

MONOCLONAL ANTIBODY

Anti-HMGB1 (HMG1) mAb

Code No.	Clone	Subclass	Quantity	Concentration
M137-3	4C9	Mouse IgG1	100 µL	1 mg/mL

BACKGROUND: High mobility group box 1 (HMGB1), named for its rapid migration properties on electrophoretic gels, is a member of the nonhistone chromatin-associated proteins. HMGB1 is translated as a 214 amino acid protein, and extensively modified posttranslationally, by glycosylation, acylation, methylation, and phosphorylation. The primary structure is evolutionarily conserved, with 100% amino acid sequence homology between rat and mouse, and 99% homology between rodent and human. Intracellular HMGB1 has been studied previously for its roles in binding DNA; stabilizing nucleosome formation; as a general transcription factor for nucleolar and mitochondrial RNA polymerases; and as a gene- and tissue-specific transcriptional regulator that can enhance transcription and/or replication. Extracellular HMGB1 is recently implicated as a late mediator of delayed endotoxin lethality, because murine and human macrophages/monocytes release large amounts of a 29 kDa form of HMGB1 when stimulated by exposure to bacterial endotoxin.

SOURCE: This antibody was purified from hybridoma (clone 4C9) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with Balb/c mouse lymphocyte immunized with the synthetic peptide corresponding to internal region of human HMGB1.

FORMULATION: 100 µg IgG in 100 µL volume of PBS containing 50% glycerol, pH 7.2. No preservative is contained.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at -20°C.

REACTIVITY: This antibody reacts with HMGB1 on Western blotting.

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	Raji, HeLa, HL-60	WR19L	Rat-1
Reactivity on WB	+	+	+

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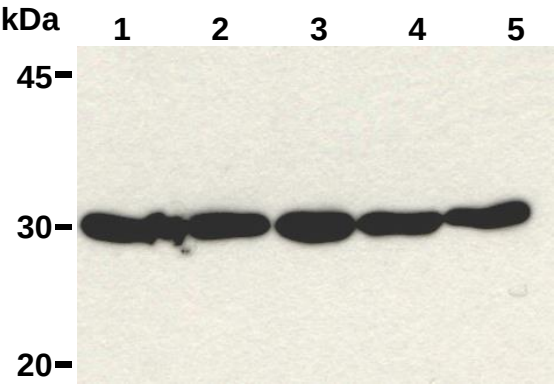
APPLICATIONS:
Western blotting; 1 µg/mL for chemiluminescence detection system
Immunoprecipitation; Not tested
Immunohistochemistry; Not tested
Immunofluorescence; Not tested*
*It is reported that this antibody can be used in Immunohistochemistry in the reference number 1).
Immunocytochemistry; Not tested
Flow cytometry; Not tested

Detailed procedure is provided in the following **PROTOCOL**.

REFERENCES:
1) Szénási, T., *et al.*, *Biochim. Biophs. Acta* **1829**, 1075-1091 (2013) [IF]
2) Taguchi, A., *et al*., *Nature* **405**, 354-360 (2000)
3) Wang, H., *et al*., *Science* **285**, 248-251 (1999)
4) Wen, L., *et al*., *Nucleic Acids Res.* **17**, 1197-1214 (1989)
5) Bianchi, M. E., *et al., Science* **243**, 1056-1059 (1989)

RELATED PRODUCTS:
D090-3 Anti-HMGB1/2 (HMG1/2) mAb (FBH7)
D075-3 Anti-HMGB1 (HMG1) mAb (KS1)



Western blot analysis of HMGB1 expression in Raji (1), HeLa (2), HL-60 (3), WR19L (4) and Rat-1 (5) using M137-3.

PROTOCOL:
SDS-PAGE & Western Blotting
1) Wash the $1x10^7$ cells 3 times with PBS and suspend with 1 mL of Laemmli's sample buffer.
2) Boil the samples for 2 minutes and centrifuge. Load 10 µL of the sample per lane in a 1 mm thick

SDS-polyacrylamide gel for electrophoresis.

3) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacture's manual for precise transfer procedure.
4) To reduce nonspecific binding, soak the membrane in 5% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
5) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggest in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
7) Incubate the membrane with the 1:10,000 Anti-IgG (Mouse) pAb-HRP (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
8) Wash the membrane with PBS-T (5 minutes x 3 times).
9) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
10) Expose to an X-ray film in a dark room for 3 minutes. Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; Raji, HeLa, HL-60, WR19L, Rat-1)