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

MONOCLONAL ANTIBODY

Anti-HMGB1 (HMG1)

Code No.	Clone	Subclass	Quantity	Concentration
D075-3	KS1	Mouse IgG2a	100 µg	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 KS1) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell PAI with Balb/c mouse lymphocyte immunized with porcine 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 (29 kDa) on Western blotting.

APPLICATIONS:

Western blotting; 5 µg/mL for chemiluminescence detection system
Immunoprecipitation; Not tested
Immunohistochemistry; Not determined
Immunocytochemistry; Not tested
Flow cytometry; Not tested

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

INTENDED USE:
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SPECIES CROSS REACTIVITY:

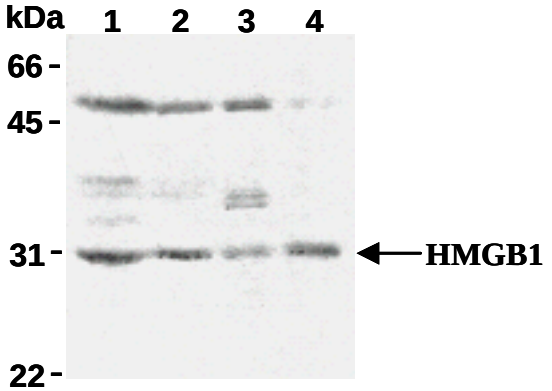
Species	Human	Mouse	Rat
Cells	Raji, HeLa, HL-60	WR19L	Rat-1, PC12
Reactivity on WB	+	+	+

REFERENCES:

1) Ito, I., *et al., J. Biol. Chem.* 10.1074/jbc.M608467200 (2007)
2) Portp, A., *et al., FASEB J.* **20**, 2565-2566 (2006)
3) Yamada, M., *et al., J. Biochem.* **135**, 14153 (2004)
4) Ito, I., *et al., J. Biochem.* **136**, 155-162 (2004)
5) Taguchi, A., *et al., Nature* **405**, 354-360 (2000)
6) Wang, H., *et al., Science* **285**, 248-251 (1999)
7) Sobajima, J., *et al., Clin. Exp. Immunol.*107, 135-140 (1997)

Clone KS1 is used in reference number 1) - 4).

RELATED PRODUCT:
D090-3 Anti-HMGB1/2 (HMG1/2) (FBH7)



Western blot analysis of HMGB1 expression in Raji (1), HL-60 (2), WR19L (3) and PC12 (4) using D075-3.

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PROTOCOL:

SDS-PAGE & Western Blotting

1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mM Tris-HCl, pH 7.2, 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol) containing appropriate protease inhibitors. Incubate it at 4^{o}C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4^{o}C and transfer the supernatant to another tube. Measure the protein concentration of the supernatant and add the Lysis buffer to make 8 mg/mL solution.
3) Mix the sample with equal volume of Laemmli's sample buffer.
4) 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.
5) 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.
6) 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^{o}C.
7) Incubate the membrane with primary antibody diluted with PBS, pH7.2 containing 1% skimmed milk as suggest in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 6 times).
9) Incubate the membrane with the 1:10,000 HRP-conjugated anti-mouse IgG (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
10) Wash the membrane with PBS-T (5 minutes x 6 times).
11) 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.
12) 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, HL-60, WR19L, PC12)