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Product datasheet for AM26447AF-N

HMG1 (HMGB1) Mouse Monoclonal Antibody [Clone ID: KS1]

Product data:

Product Type:	Primary Antibodies
Clone Name:	KS1
Applications:	WB
Recommended Dilution:	**Western blot:** 5 µg/ml for chemiluminescence detection system. For details see protocol below.
Reactivity:	Human, Mouse, Porcine, Rat
Host:	Mouse
Isotype:	IgG2a
Clonality:	Monoclonal
Immunogen:	Porcine HMGB1
Specificity:	This antibody reacts with human, mouse, rat and porcine HMGB1.
Formulation:	PBS containing 50% glycerol, pH 7.2. Contains no preservatives. State: Azide Free State: Liquid Ig fraction
Concentration:	lot specific
Purification:	Protein A agarose
Conjugation:	Unconjugated
Storage:	Store (in aliquots) at -20 °C. Avoid repeated freezing and thawing.
Stability:	Shelf life: one year from despatch.
Predicted Protein Size:	29 kDa
Gene Name:	high mobility group box 1
Database Link:	Entrez Gene 3146 Human P09429

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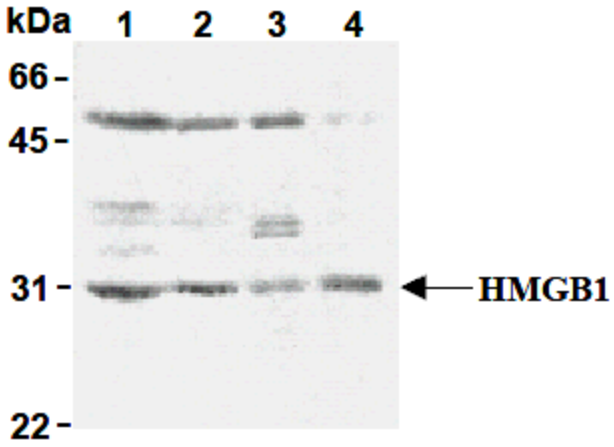
Background: High mobility group box 1 (HMGB1), named for its rapid migration properties on electrophoretic gels, is a member of the nonhistone chromatin-associated proteins. HMGB1 is translated as a 214 amino acid protein and is extensively modified posttranslationally by glycosylation, acylation, methylation, 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 previously been studied 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 has recently been 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.

Synonyms: High mobility group protein 1, HMG1, HMG-1, High mobility group protein B1, HMGB-1, Amphoterin

Note: This product was originally produced by MBL International.

Protocol:
SDS-PAGE & Western Blotting
1) Wash the cells 3 times with PBS and suspend with 10 volumes 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 4oC 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 4oC 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 an 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/cm2 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 4oC.
7) Incubate the membrane with primary antibody diluted with PBS, pH7.2 containing 1% skimmed milk as suggested 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 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 from the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute. Remove extra reagent from the membrane by dabbing with a 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 conditions for exposure and development may vary.
(Positive controls for Western blotting; Raji, HL-60, WR19L, PC12)

Product images:



Western blot analysis of HMGB1 expression in Raji (1), HL-60 (2), WR19L (3) and PC12 (4) using AM26447AF-N.