

## Product datasheet for **AM26643AF-N**

### HMGB1 (HMGB1) Mouse Monoclonal Antibody [Clone ID: 4C9]

#### Product data:

Product Type:	Primary Antibodies
Clone Name:	4C9
Applications:	WB
Recommended Dilution:	Western blot: 1 µg/ml for chemiluminescence detection system. For details see protocol below.
Reactivity:	Human, Mouse, Rat
Host:	Mouse
Isotype:	IgG
Clonality:	Monoclonal
Immunogen:	Synthetic peptide corresponding to internal region of human HMGB1
Specificity:	This antibody reacts with HMGB1.
Formulation:	PBS containing 50% glycerol, pH 7.2. No preservative is contained. State: Azide Free State: Liquid Ig fraction
Concentration:	lot specific
Purification:	Protein A agarose
Conjugation:	Unconjugated
Storage:	Upon receipt, store (in aliquots) at -20 °C. Avoid repeated freezing and thawing.
Stability:	Shelf life: One year from despatch.
Gene Name:	high mobility group box 1
Database Link:	<a href="#">Entrez Gene 3146 Human P09429</a>

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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 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.

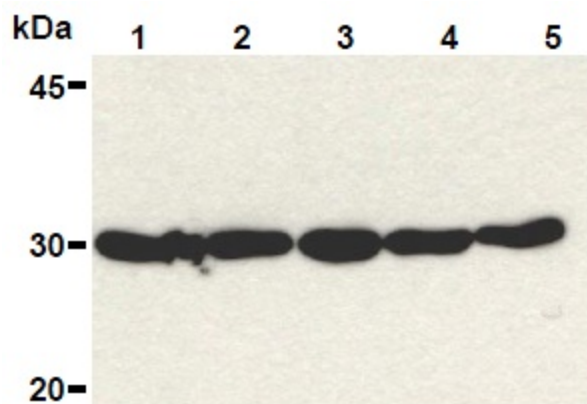
**Synonyms:** High mobility group protein 1, HMG1, HMG-1, High mobility group protein B1, HMGB-1, Amphoterin

**Note:** This product was originally produced by MBL International.

**Protocol:**

**SDS-PAGE & Western Blotting**

- 1) Wash the 1x10<sup>7</sup> 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<sup>2</sup> 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 HRP-conjugated anti-mouse IgG 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)

**Product images:**

Western blot analysis of HMGB1 expression in Raji (1), HeLa (2), HL-60 (3), WR19L (4) and Rat-1 (5) using AM26643AF-N.