OriGene Technologies, Inc.
9620 Medical Center Drive, Ste 200
Rockville, MD 20850, US
Phone: +1-888-267-4436
https://www.origene.com
techsupport@origene.com
EU: info-de@origene.com
CN: techsupport@origene.cn

Product datasheet for AM26643AF-N

HMG1 (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 aliqouts) at -20 °C. Avoid repeated freezing and thawing.
Stability:	Shelf life: One year from despatch.
Gene Name:	high mobility group box 1
Database Link:	Entrez Gene 3146 Human P09429

View online »

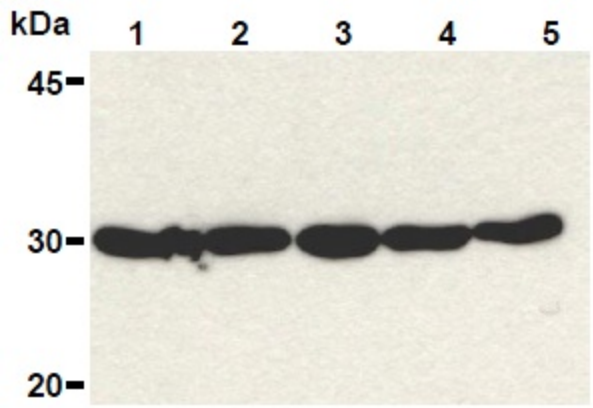
Background: High mobility group box 1 (HMGB1), named for its rapid migration properties on electrophoretic gels, is a member of the nonhistone chromatin-associated proteins. HMGB1 is translated as a 214 amino acid protein, and extensively modified posttranslationally, by glycosylation, acylation, methylation, 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 1x10e7 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/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.
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 4oC.
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.