

**Anti-HMG4 Antibody Picoband™ (monoclonal, 8H9)**  
Catalog # ABO14923**Specification****Anti-HMG4 Antibody Picoband™ (monoclonal, 8H9) - Product Information**

Application	WB, IF, ICC, FC
Primary Accession	<a href="#">O15347</a>
Host	Mouse
Isotype	Mouse IgG2b
Reactivity	Rat, Human, Mouse
Clonality	Monoclonal
Format	Lyophilized

**Description**

Anti-HMG4 Antibody Picoband™ (monoclonal, 8H9) . Tested in Flow Cytometry, IF, ICC, WB applications. This antibody reacts with Human, Mouse, Rat.

**Reconstitution**

Add 0.2ml of distilled water will yield a concentration of 500 µg/ml.

**Anti-HMG4 Antibody Picoband™ (monoclonal, 8H9) - Additional Information**

**Gene ID** 3149

**Other Names**

High mobility group protein B3, High mobility group protein 2a, HMG-2a, High mobility group protein 4, HMG-4, HMGB3, HMG2A, HMG4

**Calculated MW**

23 kDa KDa

**Application Details**

Western blot, 0.1-0.5 µg/ml, Human, Mouse, Rat  
Immunocytochemistry/Immunofluorescence, 2 µg/ml, Human  
Flow Cytometry, 1-3 µg/1x10<sup>6</sup> cells, Human

**Subcellular Localization**

Nucleus. Chromosome. Cytoplasm.

**Tissue Specificity**

Expressed predominantly in placenta.

**Protein Name**

High mobility group protein B3

**Contents**

Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na<sub>2</sub>HPO<sub>4</sub>, 0.05mg Na<sub>3</sub>N.

**Immunogen**

A synthetic peptide corresponding to a sequence at the N-terminus of human HMG4, identical to the related mouse and rat sequences.

**Purification**

Immunogen affinity purified.

**Cross Reactivity**

No cross-reactivity with other proteins.

**Storage**

Store at  $-20^{\circ}\text{C}$  for one year from date of receipt. After reconstitution, at  $4^{\circ}\text{C}$  for one month. It can also be aliquotted and stored frozen at  $-20^{\circ}\text{C}$  for six months. Avoid repeated freeze-thaw cycles.

**Anti-HMG4 Antibody Picoband™ (monoclonal, 8H9) - Protein Information**

**Name** HMGB3

**Synonyms** HMG2A, HMG4

**Function**

Multifunctional protein with various roles in different cellular compartments. May act in a redox sensitive manner. Associates with chromatin and binds DNA with a preference for non-canonical DNA structures such as single-stranded DNA. Can bend DNA and enhance DNA flexibility by looping thus providing a mechanism to promote activities on various gene promoters (By similarity). Proposed to be involved in the innate immune response to nucleic acids by acting as a cytoplasmic promiscuous immunogenic DNA/RNA sensor (By similarity). Negatively regulates B-cell and myeloid cell differentiation. In hematopoietic stem cells may regulate the balance between self-renewal and differentiation. Involved in negative regulation of canonical Wnt signaling (By similarity).

**Cellular Location**

Nucleus {ECO:0000250|UniProtKB:P40618, ECO:0000255|PROSITE-ProRule:PRU00267}.  
Chromosome Cytoplasm {ECO:0000250|UniProtKB:O54879}

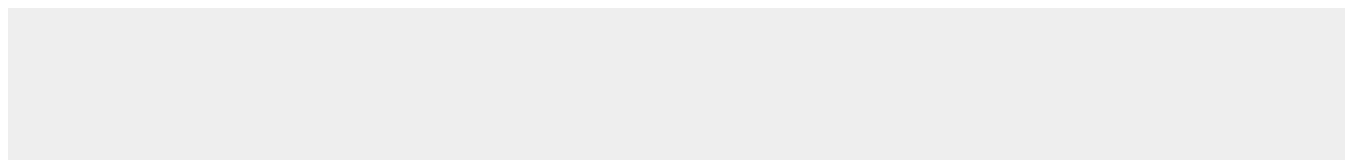
**Tissue Location**

Expressed predominantly in placenta.

**Anti-HMG4 Antibody Picoband™ (monoclonal, 8H9) - Protocols**

Provided below are standard protocols that you may find useful for product applications.

- [Western Blot](#)
- [Blocking Peptides](#)
- [Dot Blot](#)
- [Immunohistochemistry](#)
- [Immunofluorescence](#)
- [Immunoprecipitation](#)
- [Flow Cytometry](#)
- [Cell Culture](#)

**Anti-HMG4 Antibody Picoband™ (monoclonal, 8H9) - Images**

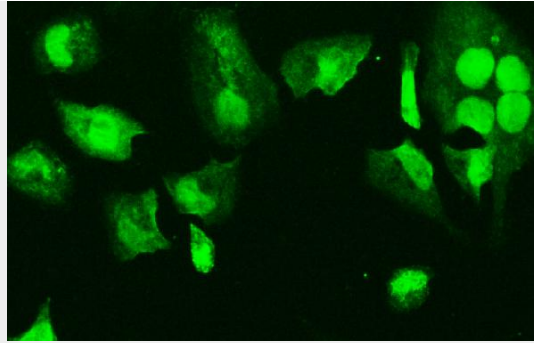


Figure 3. IF analysis of HMGB3 using anti-HMGB3 antibody (M02834-1). HMGB3 was detected in immunocytochemical section of HeLa cell. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 2 µg/mL mouse anti-HMGB3 Antibody (M02834-1) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Mouse IgG (BA1126) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

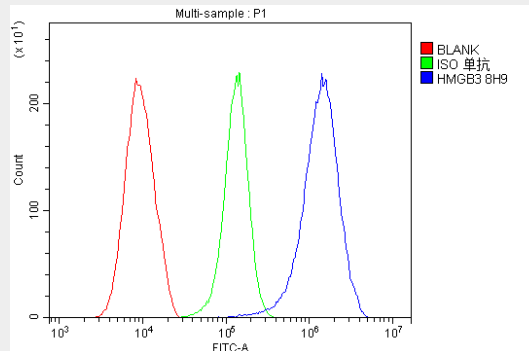


Figure 4. Flow Cytometry analysis of HeLa cells using anti-HMGB3 antibody (M02834-1). Overlay histogram showing HeLa cells stained with M02834-1 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-HMGB3 Antibody (M02834-1, 1 µg/1x10<sup>6</sup> cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 µg/1x10<sup>6</sup> cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 µg/1x10<sup>6</sup>) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

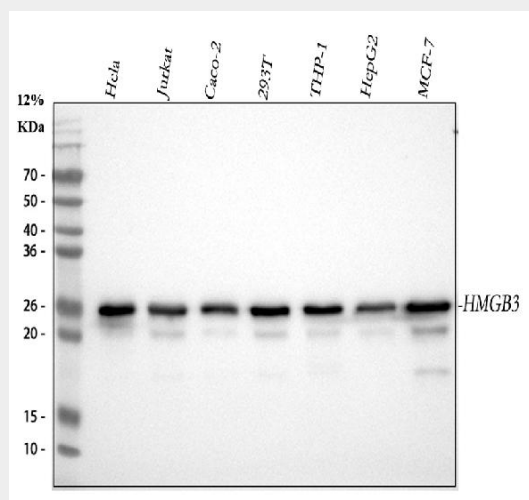


Figure 1. Western blot analysis of HMGB3 using anti-HMGB3 antibody (M02834-1).

Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30ug of sample under reducing conditions.

- Lane 1: human Hela whole cell lysates,
- Lane 2: human Jurkat whole cell lysates,
- Lane 3: human CACO-2 whole cell lysates,
- Lane 4: human 293T whole cell lysates,
- Lane 5: human THP-1 whole cell lysates,
- Lane 6: human HepG2 whole cell lysates,
- Lane 7: human MCF-7 whole cell lysates.

After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA for 50-90 minutes. Blocked the membrane with 5% Non-fat Milk/ TBS for 1.5 hour at RT. The membrane was incubated with mouse anti-HMGB3 antigen affinity purified monoclonal antibody (Catalog # M02834-1) at 0.25 µg/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-mouse IgG-HRP secondary antibody at a dilution of 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1001) with Tanon 5200 system. A specific band was detected for HMGB3 at approximately 23 kDa. The expected band size for HMGB3 is at 23 kDa.

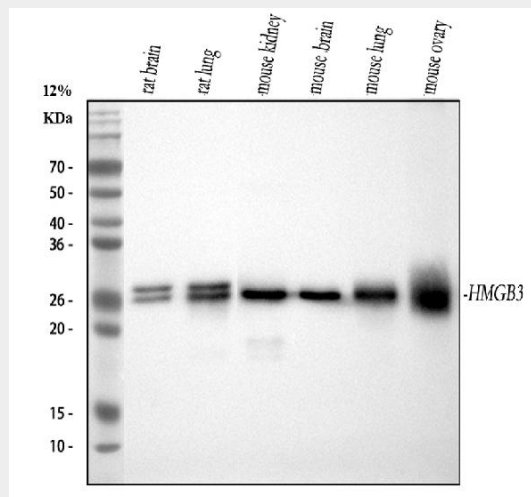


Figure 2. Western blot analysis of HMGB3 using anti-HMGB3 antibody (M02834-1).

Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30ug of sample under reducing conditions.

- Lane 1: rat brain tissue lysates,
- Lane 2: rat lung tissue lysates,
- Lane 3: mouse kidney tissue lysates,
- Lane 4: mouse brain tissue lysates,
- Lane 5: mouse lung tissue lysates,
- Lane 6: mouse ovary tissue lysates.

After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA for 50-90 minutes. Blocked the membrane with 5% Non-fat Milk/ TBS for 1.5 hour at RT. The membrane was incubated with mouse anti-HMGB3 antigen affinity purified monoclonal antibody (Catalog # M02834-1) at 0.25 µg/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-mouse IgG-HRP secondary antibody at a dilution of 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1001) with Tanon 5200 system. A specific band was detected for HMGB3 at approximately 23 kDa. The expected band size for HMGB3 is at 23 kDa.

**Anti-HMG4 Antibody Picoband™ (monoclonal, 8H9) - Background**

High-mobility group protein B, also known as HMG4, is a protein that in humans is encoded by the HMGB3 gene. This gene encodes a member of a family of proteins containing one or more high mobility group DNA-binding motifs. The encoded protein plays an important role in maintaining stem cell populations, and may be aberrantly expressed in tumor cells. A mutation in this gene was associated with microphthalmia, syndromic 13. There are numerous pseudogenes of this gene on multiple chromosomes. Alternative splicing results in multiple transcript variants.