

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

Catalog # ABO14923

## Product Information

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|--------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Application</b>       | WB, IF, ICC, FC                                                                                                                                   |
| <b>Primary Accession</b> | <a href="#">O15347</a>                                                                                                                            |
| <b>Host</b>              | Mouse                                                                                                                                             |
| <b>Isotype</b>           | Mouse IgG2b                                                                                                                                       |
| <b>Reactivity</b>        | Rat, Human, Mouse                                                                                                                                 |
| <b>Clonality</b>         | Monoclonal                                                                                                                                        |
| <b>Format</b>            | Lyophilized                                                                                                                                       |
| <b>Description</b>       | Anti-HMG4 Antibody Picoband™ (monoclonal, 8H9) . Tested in Flow Cytometry, IF, ICC, WB applications. This antibody reacts with Human, Mouse, Rat. |
| <b>Reconstitution</b>    | Add 0.2ml of distilled water will yield a concentration of 500 µg/ml.                                                                             |

## Additional Information

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| <b>Gene ID</b>                  | 3149                                                                                                                                                              |
| <b>Other Names</b>              | High mobility group protein B3, High mobility group protein 2a, HMG-2a, High mobility group protein 4, HMG-4, HMGB3, HMG2A, HMG4                                  |
| <b>Calculated MW</b>            | 22980                                                                                                                                                             |
| <b>Application Details</b>      | Western blot, 0.1-0.5 µg/ml, Human, Mouse, Rat<br>Immunocytochemistry/Immunofluorescence, 2 µg/ml, Human<br>Flow Cytometry, 1-3 µg/1x10 <sup>6</sup> cells, Human |
| <b>Subcellular Localization</b> | Nucleus. Chromosome. Cytoplasm.                                                                                                                                   |
| <b>Tissue Specificity</b>       | Expressed predominantly in placenta.                                                                                                                              |
| <b>Source</b>                   | Eukaryota                                                                                                                                                         |
| <b>Protein Name</b>             | High mobility group protein B3                                                                                                                                    |
| <b>Contents</b>                 | Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na <sub>2</sub> HPO <sub>4</sub> , 0.05mg NaN <sub>3</sub> .                                                  |
| <b>Clone Names</b>              | Clone: 8H9                                                                                                                                                        |
| <b>Immunogen</b>                | A synthetic peptide corresponding to a sequence at the N-terminus of human HMG4, identical to the related mouse and rat sequences.                                |
| <b>Purification</b>             | Immunogen affinity purified.                                                                                                                                      |

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|-------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Cross Reactivity</b> | No cross-reactivity with other proteins.                                                                                                                                                                 |
| <b>Storage</b>          | Store at -20 °C for one year from date of receipt. After reconstitution, at 4 °C for one month. It can also be aliquotted and stored frozen at -20 °C for six months. Avoid repeated freeze-thaw cycles. |

## Protein Information

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| <b>Name</b>              | HMGB3                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| <b>Synonyms</b>          | HMG2A, HMG4                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| <b>Function</b>          | 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). |
| <b>Cellular Location</b> | Nucleus {ECO:0000250 UniProtKB:P40618, ECO:0000255 PROSITE-ProRule:PRU00267}. Chromosome Cytoplasm {ECO:0000250 UniProtKB:O54879}                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| <b>Tissue Location</b>   | Expressed predominantly in placenta.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |

## 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.

## Images

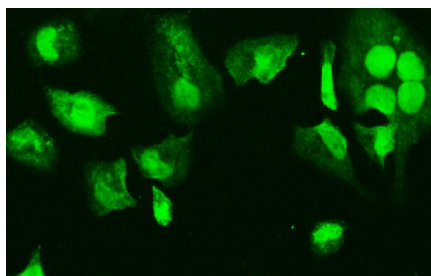


Figure 3. IF analysis of HMGB3 using anti-HMGB3 antibody (M02834-1). HMGB3 was detected in immunocytochemical section of HeLa cell. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 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.

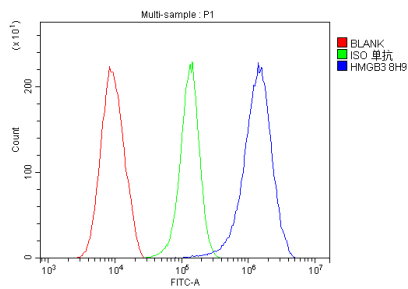


Figure 4. Flow Cytometry analysis of Hela cells using anti-HMGB3 antibody (M02834-1). Overlay histogram showing Hela cells stained with M02834-1 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-HMGB3 Antibody (M02834-1, 1  $\mu$ g/ $1 \times 10^6$  cells)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

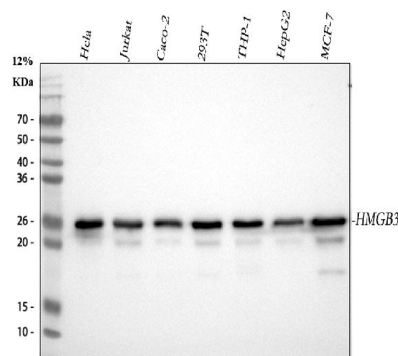


Figure 1. Western blot analysis of HMGB3 using anti-HMGB3 antibody (M02834-1). Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30ug of sample under reducing conditions. Lane 1: human Hela whole 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  $\mu$ g/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.

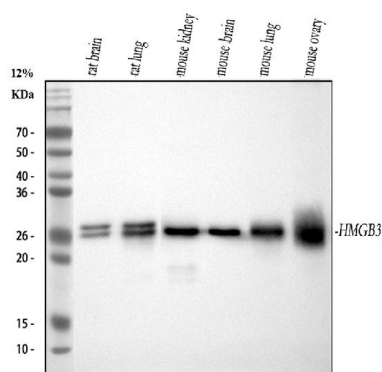


Figure 2. Western blot analysis of HMGB3 using anti-HMGB3 antibody (M02834-1). Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30ug of sample under reducing conditions. Lane 1: rat brain tissue 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  $\mu$ g/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.

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.