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

Catalog # ABO14923

Specification

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

Application	**WB, IF, ICC, FC**
Primary Accession	O15347
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/1x10^6 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 Na2HPO4, 0.05mg NaN3.

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˚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.**

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 Cytomety
- Cell Culture

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

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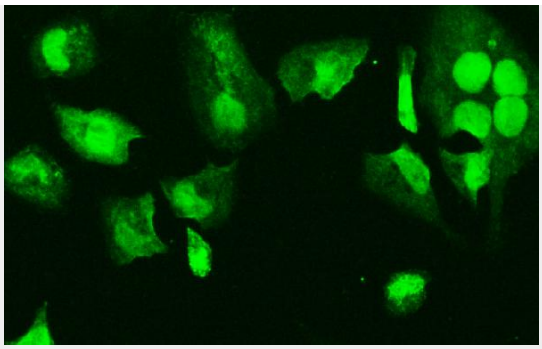

Figure 3. IF analysis of HMGB3 using anti-HMGB3 antibody (M02834-1).
HMGB3 was detected in immunocytochemical section of Hela cell. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with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

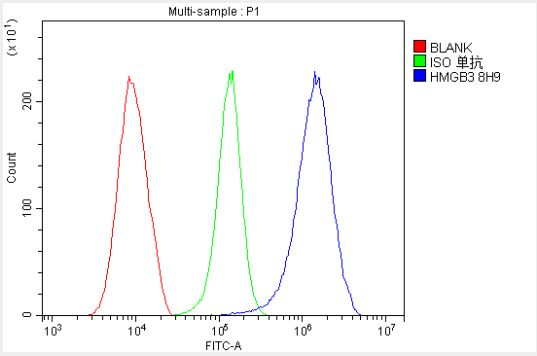

Figure 4. Flow Cytometry analysis of Hela cells using anti-HMGB3 antibody (M02834-1).
Overlay histogram showing Hela cells stained with M02834-1 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-HMGB3 Antibody (M02834-1, 1 µg/1x10^6 cells) for 30 min at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

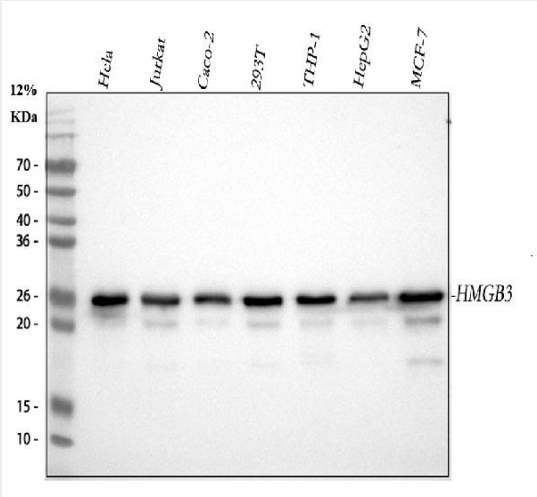

Figure 1. Western blot analysis of HMGB3 using anti-HMGB3 antibody (M02834-1).

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 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.

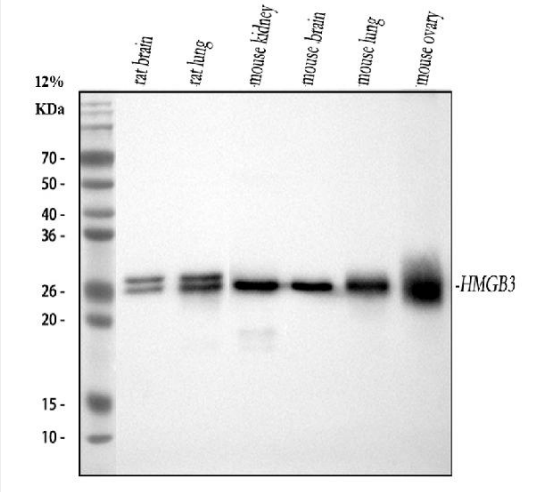

Figure 2. Western blot analysis of HMGB3 using anti-HMGB3 antibody (M02834-1).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30ug of sample under reducing conditions.
Lane 1: rat brain tissue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