

Anti-HMG4 Antibody Picoband™ (monoclonal, 7G13)

Catalog # ABO14922

Product Information

Application	WB, IHC, IF, ICC, FC
Primary Accession	O15347
Host	Mouse
Isotype	Mouse IgG2b
Reactivity	Human
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-HMG4 Antibody Picoband™ (monoclonal, 7G13) . Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human.
Reconstitution	Add 0.2ml of distilled water will yield a concentration of 500 µg/ml.

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	22980
Application Details	Western blot, 0.1-0.5 µg/ml, Human Immunohistochemistry (Paraffin-embedded Section), 0.5-1 µg/ml, Human, 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.
Source	Eukaryota
Protein Name	High mobility group protein B3
Contents	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na ₂ HPO ₄ , 0.05mg NaN ₃ .
Clone Names Immunogen	Clone: 7G13 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.

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.

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

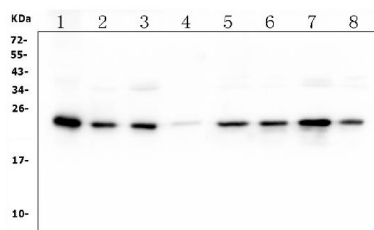


Figure 1. Western blot analysis of HMGB3 using anti-HMGB3 antibody (M02834). Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions. Lane 1: human placenta tissue lysates, Lane 2: human HeLa whole cell lysates, Lane 3: human T-47D whole cell lysates, Lane 4: human A431 whole cell lysates, Lane 5: human HepG2 whole cell lysates, Lane 6: human Caco-2 whole cell lysates. Lane 7: human SW620 whole cell lysates. Lane 8: human Raji 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 polyclonal antibody (Catalog # M02834) at 0.5 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 23KD. The expected band size for HMGB3 is at 23KD.

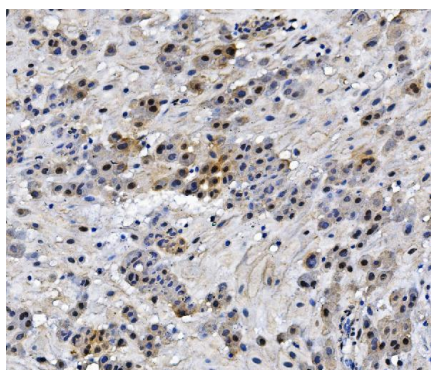


Figure 2. IHC analysis of HMGB3 using anti-HMGB3 antibody (M02834). HMGB3 was detected in paraffin-embedded section of human placenta tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-HMGB3 Antibody (M02834) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

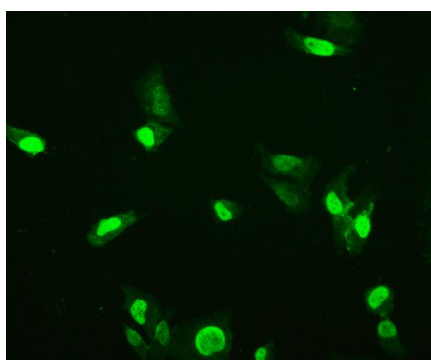


Figure 3. IF analysis of HMGB3 using anti-HMGB3 antibody (M02834). HMGB3 was detected in immunocytochemical section of HeLa cell. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 2 µg/mL mouse anti-HMGB3 Antibody (M02834) 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.

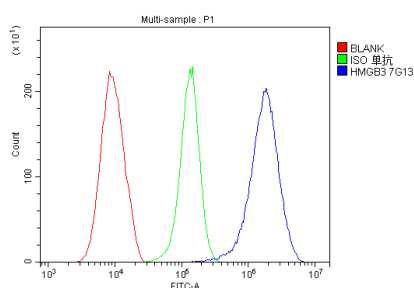


Figure 4. Flow Cytometry analysis of HeLa cells using anti-HMGB3 antibody M02834). Overlay histogram showing HeLa cells stained with M02834 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-HMGB3 Antibody (M02834, 1 µg/1x10⁶ cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 µg/1x10⁶ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 µg/1x10⁶) used under the same conditions. Unlabelled sample (Red line) was also used as a control.