Myosin Heavy Chain Antibody

Monoclonal Mouse IgG_{2B} Clone # MF20

Catalog Number: MAB4470

DESCRIPTION

Specificity	Detects Myosin Heavy Chain in human, mouse, rat and other mammalian, avian, and amphibian species.
Source	Monoclonal Mouse IgG_{2B} Clone # MF20
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	Chicken pectoralis-derived Myosin
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 µm filtered solution in PBS.

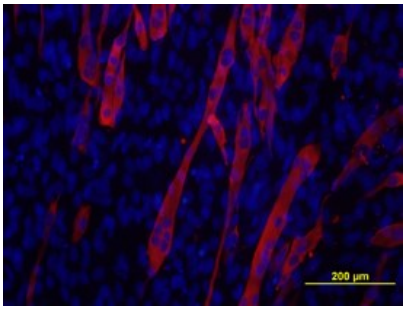
APPLICATIONS

Please Note: *Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.*

	Recommended Concentration	Sample
Flow Cytometry	0.25 µg/10^6 cells	C2C12 mouse myoblast cell line
Immunocytochemistry	8-25 µg/mL	See Below
Immunohistochemistry	5-25 µg/mL	See Below
Western Blot	Stains, C.I., *et al*. (2012) Chem Biol. **19**:210.	

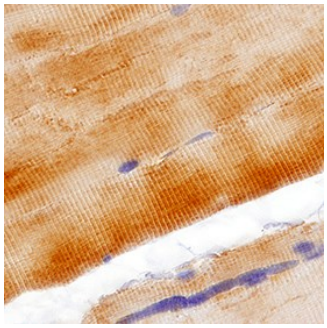
DATA

Immunocytochemistry



Myosin Heavy Chain in C2C12 Mouse Cell Line. Myosin Heavy Chain was detected in immersion fixed C2C12 mouse myoblast cell line using Mouse Anti-Human Myosin Heavy Chain Monoclonal Antibody (Catalog # MAB4470) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Mouse IgG Secondary Antibody (red; Catalog # Catalog # NL007) and counter-stained with DAPI (blue). View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

Immunohistochemistry



Myosin Heavy Chain in Human Skeletal Muscle. Myosin Heavy Chain was detected in immersion fixed paraffin-embedded sections of human skeletal muscle using Mouse Anti-Myosin Heavy Chain Monoclonal Antibody (Catalog # MAB4470) at 5 µg/mL for 1 hour at room temperature followed by incubation with the Anti-Mouse IgG VisUCyte™ HRP Polymer Antibody (Catalog # Catalog # VC001). Before incubation with the primary antibody, tissue was subjected to heat-induced epitope retrieval using Antigen Retrieval Reagent-Basic (Catalog # Catalog # CTS013). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to sarcoplasm. View our protocol for IHC Staining with VisUCyte HRP Polymer Detection Reagents.

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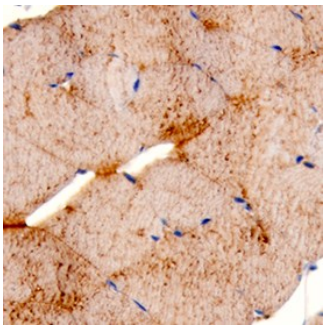
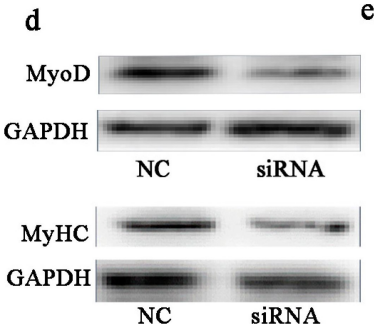
Immunohistochemistry



Myosin Heavy Chain in Mouse Skeletal Muscle. Myosin Heavy Chain was detected in perfusion fixed frozen sections of mouse skeletal muscle using Mouse Anti-Myosin Heavy Chain Monoclonal Antibody (Catalog # MAB4470) at 5 µg/mL for 1 hour at room temperature followed by incubation with the Anti-Mouse IgG VisUCyte™ HRP Polymer Antibody (Catalog # Catalog # VC001). Before incubation with the primary antibody, tissue was subjected to heat-induced epitope retrieval using Antigen Retrieval Reagent-Basic (Catalog # Catalog # CTS013). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to sarcoplasm. View our protocol for IHC Staining with VisUCyte HRP Polymer Detection Reagents.

Western Blot



Detection of Mouse Myosin Heavy Chain by Western Blot Knockdown of BAMBI inhibited myogenic differentiation. All the cell samples were harvested after transfection and myogenic induction for 48 and 96 h. (a) The western blot images of BAMBI and GAPDH; (b) the efficiency of siRNA interference on the mRNA and protein expression of BAMBI; (c) the mRNA expression of MyoD at 48 h and that of MyoG and MyHC at 96 h; (d) the western blot images of MyoD at 48 h, MyHC at 96 h, and their corresponding GAPDH; (e) the protein expression of MyoD at 48 h and MyHC at 96 h; (f) immunofluorescence of MyHC in C2C12 myotubes at 96 h post differentiation, images captured at 100× magnification; (g) the populations of myotubes; (h) the differentiation index; and (i) the myotube fusion index. The results were represented as mean ± SD; n = 3; * $p < 0.05$; ** $p < 0.01$. Image collected and cropped by CiteAb from the following publication (https://www.mdpi.com/1422-0067/16/8/17734), licensed under a CC-BY license. Not internally tested by R&D Systems.

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Myosin Heavy Chain Antibody

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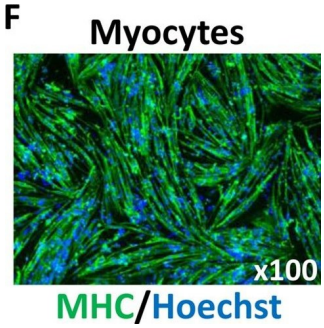
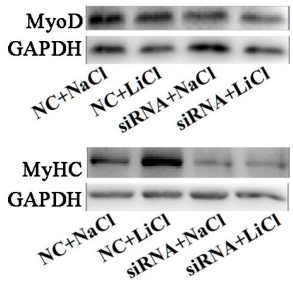
Immunocytochemistry/ Immunofluorescence



Detection of Human Myosin Heavy Chain by Immunocytochemistry/Immunofluorescence Generation of DM1-iPSCs and their differentiation.(A) The strategy of our study: patient iPSCs were passed and differentiated at three different passage numbers into CMs or neurons giving 9 samples (left), or had a MyoD1 vector transfected and were differentiated into myocytes, giving 6 samples (right). The CTG repeat lengths were measured in each sample. (B) Six clones from three different DM1 patients expressed pluripotent stem cell markers (Oct3/4, Nanog and Sox2) in conventional PCR. β-actin was used as a loading control. (C) Karyotypic analysis of undifferentiated iPSCS (Pt-1B). (D, left) Representative live image of CMs on day 20 (Pt-1B). A video clip is available in Supplementary Video 1. (D, right) FACS analysis of the CMs shown in the picture on the left. The X-axis indicates the percentage of cardiac troponin T (cTnT)-positive cells among the total number of CMs. The Y-axis indicates the autofluorescence of the CMs. (E) Representative immunostaining image of neurons on day 42 (Pt-1B). The left panel shows neurons that expressed Tyrosine Hydroxylase (TH) and Microtubule-associated protein 2 (Map2). The right panel shows neurons that expressed TH and Neuron-specific Class III β-tubulin (TUJ1). (F) Representative immunostaining image of myocytes on day 7 (Pt-1B). The myocytes expressed Myosin Heavy Chain (MHC). Hoechst stains the nuclei. Image collected and cropped by CiteAb from the following publication (https://www.nature.com/articles/srep42522), licensed under a CC-BY license. Not internally tested by R&D Systems.

Western Blot



Detection of Mouse Myosin Heavy Chain by Western Blot LiCl rescued the inhibitory effect of BAMBI siRNA on C2C12 myogenic differentiation. All the cell samples were harvested after transfection and myogenic induction for 48 and 96 h. (a) The mRNA expression of MyoD at 48 h and that of MyoG and MyHC at 96 h; (b) the western blot images of MyoD, MyHC, and GAPDH; (c) the protein expression of MyoD at 48 h and MyHC at 96 h; (d) immunofluorescence images of MyHC in C2C12 myotubes at 96 h post differentiation, images captured at 100× magnification; (e) the populations of myotubes; (f) the differentiation index and (g) the myotube fusion index. The results were represented as mean ± SD; n = 3; * $p < 0.05$; ** $p < 0.01$. Image collected and cropped by CiteAb from the following publication (https://www.mdpi.com/1422-0067/16/8/17734), licensed under a CC-BY license. Not internally tested by R&D Systems.

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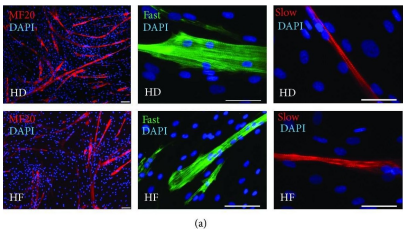
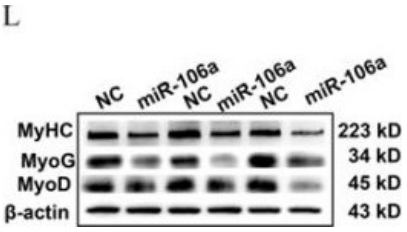


(a)

Detection of Human Myosin Heavy Chain by Immunocytochemistry/Immunofluorescence Differentiated myotubes do not differ significantly between HD- and HF-derived skeletal muscle progenitor cells. (a) At day 7 after stimulation, myotubes were stained for the expression of MyHC with an antibody that recognizes the heavy chain of myosin II (MF20) and markers of slow MYH7 and fast MYH1/MYH2 fibers. Nuclei were labelled with DAPI (blue). Representative images are given for both HF- and HD-derived samples. Scale bars represent 50 µm. (b) Fusion coefficient is calculated as a percent of nuclei incorporated in MF20+ myotubes at day 7 after stimulation, and it does not differ between HD- and HF-derived samples. (c) mRNA expression analysis was performed for key markers of muscle development and metabolism for both HF- and HD-derived samples. Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/30719048), licensed under a CC-BY license. Not internally tested by R&D Systems.

Western Blot



Detection of Mouse Myosin Heavy Chain by Western Blot MiR-106a-5p inhibited the myogenic differentiation of C2C12 myoblasts. (A) Overexpression efficiency of miR-106a-5p 3 days (d) and 5 d post differentiation. NC: negative control; (B) The fluorescent microscopy images of C2C12 cells transfected with FAM-labeled miR-106a-5p agomir (×10). Scale bars = 500 µm; (C) Immunostaining for MyHC (red) and DAPI (blue) on 5 d post differentiation (×20). Scale bars = 100 µM; (D–F) The statistical results of differentiation index, fusion index and the populations of myotubes, respectively;1-3 indicates myotubes with 1, 2 or 3 nucleus, >4 indicates myotubes with 4 more nucleus; (G,H) The mRNA expression of MyoD, MyoG, MyHC on 3 d and 5 d post differentiation; (I,J) The mRNA expression of Myomarker and Myomixer 3 d and 5 d post differentiation; (K) The statistical results of MyoD, MyoG, MyHC proteins in Figure 2L; (L) Western blot analyzed for MyoD, MyoG, MyHC proteins 5 d post differentiation; (M) Protein levels of key molecules in PI3K-AKT pathway in C2C12 cells transfected with miR-106a-5p agomir or NC on 5 d post differentiation; (N) The statistical analysis of phosphorylated PI3K (p85α), AKT (sre473) and mTOR (ser2448). Data were presented as mean ± SEM. n = 3 per group. * $p < 0.05$, ** $p < 0.01$. Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/30004470), licensed under a CC-BY license. Not internally tested by R&D Systems.

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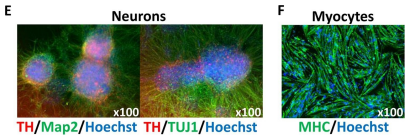

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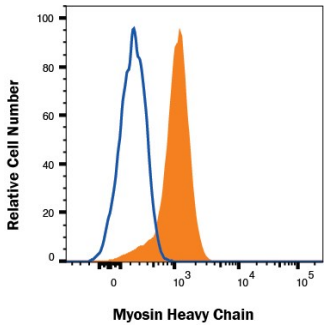
Generation of DM1-iPSCs and their differentiation.(A) The strategy of our study: patient iPSCs were passed and differentiated at three different passage numbers into CMs or neurons giving 9 samples (left), or had a MyoD1 vector transfected and were differentiated into myocytes, giving 6 samples (right). The CTG repeat lengths were measured in each sample. (B) Six clones from three different DM1 patients expressed pluripotent stem cell markers (Oct3/4, Nanog and Sox2) in conventional PCR. β-actin was used as a loading control. (C) Karyotypic analysis of undifferentiated iPSCS (Pt-1B). (D, left) Representative live image of CMs on day 20 (Pt-1B). A video clip is available in Supplementary Video 1. (D, right) FACS analysis of the CMs shown in the picture on the left. The X-axis indicates the percentage of cardiac troponin T (cTnT)-positive cells among the total number of CMs. The Y-axis indicates the autofluorescence of the CMs. (E) Representative immunostaining image of neurons on day 42 (Pt-1B). The left panel shows neurons that expressed Tyrosine Hydroxylase (TH) and Microtubule-associated protein 2 (Map2). The right panel shows neurons that expressed TH and Neuron-specific Class III β-tubulin (TUJ1). (F) Representative immunostaining image of myocytes on day 7 (Pt-1B). The myocytes expressed Myosin Heavy Chain (MHC). Hoechst stains the nuclei. Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/28211918), licensed under a CC-BY license. Not internally tested by R&D Systems.

Flow Cytometry



Detection of Myosin Heavy Chain in C2C12 cells by Flow Cytometry C2C12 cells were stained with Mouse Anti-Myosin Heavy Chain Monoclonal Antibody (Catalog # mab4470, filled histogram) or isotype control antibody (Catalog # MAB004, open histogram) followed by Allophycocyanin-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # F0101B). To facilitate intracellular staining, cells were fixed with Flow Cytometry Fixation Buffer (Catalog # FC004) and permeabilized with Saponin. View our protocol for Staining Intracellular Molecules.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.5 mg/mL in sterile PBS. For liquid material, refer to CoA for concentration.
Shipping	Lyophilized product is shipped at ambient temperature. Liquid small pack size (-SP) is shipped with polar packs. Upon receipt, store immediately at the temperature recommended below.
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** • 12 months from date of receipt, -20 to -70 °C as supplied. • 1 month, 2 to 8 °C under sterile conditions after reconstitution. • 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

Skeletal muscle Myosin or myosin II is the motor protein that generates force to drive muscle contraction. It is a 520 kDa hexamer comprised of two heavy chains and four light chains. Myosin heavy chain is 220 kDa in size and consists of a long coiled-coil domain tail that mediates dimerization of the two heavy chains and a globular head region that mediates ATP-dependent sliding of actin filaments. Myosin heavy chain can be proteolytically cleaved to produce heavy meromyosin, which includes the S1 motor domain (head region) and first third of the coiled coil domain, and light meromyosin, which includes the C-terminal two thirds of the coiled coil domain.

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