

HK2 Monoclonal Antibody (3D3)

Catalog NumberMA5-15679

Product data sheet

Details		Species Reactivity	
Size	100 µL	Species reactivity	Human
Host/Isotope	Mouse / IgG1	Published species	Human, Not Applicable
Class	Monoclonal	Tested Applications	
Type	Antibody	ELISA (ELISA)	Dilution *1:10,000
Clone	3D3	Flow Cytometry (Flow)	1:200-1:400
Immunogen	Purified recombinant fragment of human HK2 expressed in E. Coli.	Immunohistochemistry (Paraffin) (IHC (P))	1:200-1:1,000
Conjugate	Unconjugated	Western Blot (WB)	1:500-1:2,000
Form	Liquid	Immunocytochemistry (ICC/IF)	1:100
Concentration	Conc. Not Determined	* Suggested working dilutions are given as a guide only. It is recommended that the user titrate the product for use in their own experiment using appropriate negative and positive controls.	
Storage buffer	ascites		
Contains	0.03% sodium azide		
Storage Conditions	Store at 4°C short term. For long term storage, store at -20°C, avoiding freeze/thaw cycles.		

Product specific information

MA5-15679 targets HK2 in indirect ELISA, FACS, IHC and WB applications and shows reactivity with Human samples. The MA5-15679 immunogen is purified recombinant fragment of human HK2 expressed in E. Coli. MA5-15679 detects HK2 which has a predicted molecular weight of approximately 102kDa.

Background/Target Information

In vertebrates there are four major glucose-phosphorylating isoenzymes, designated hexokinase I, II, III, and IV. Hexokinase activity is involved in the first step in several metabolic pathways including phosphorylation of glucose to produce glucose-6-phosphate, thus committing glucose to the glycolytic pathway. Hexokinase is an allosteric enzyme inhibited by its product GLC-6-P. Hexokinase 2 is the predominant hexokinase isozyme expressed in insulin-responsive tissues such as skeletal muscle. Expression of this gene is insulin-responsive, and studies in rat suggest that it is involved in the increased rate of glycolysis seen in rapidly growing cancer cells.

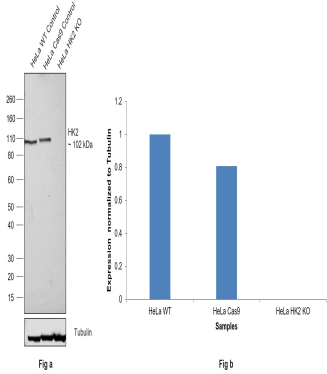
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Product Images For HK2 Monoclonal Antibody (3D3)

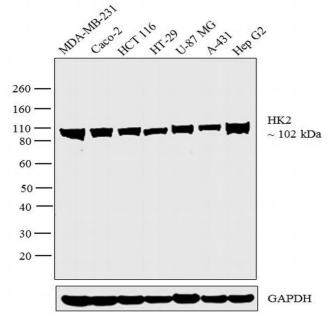


HK2 Antibody (MA5-15679)

Antibody specificity was demonstrated by CRISPR-Cas9 mediated knockout of target protein. A loss of signal was observed for target protein in HK2 KO cell line compared to control cell line using HK2 Monoclonal Antibody (3D3) (Product # MA5-15679). {KO}

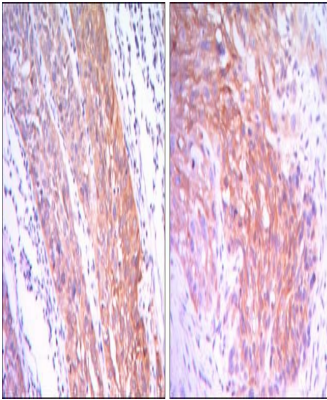
HK2 Antibody (MA5-15679) in WB

Western blot analysis was performed on whole cell extracts (30 µg lysate) of MDA-MB-231 (Lane 1), Caco-2 (Lane 2), HCT 116 (Lane 3), HT-29 (Lane 4), U-87 MG (Lane 5), A-431 (Lane 6) and Hep G2 (Lane 7). The blot was probed with Anti-HK2 Monoclonal Antibody (Product # MA5-15679, 1:1000 dilution) and detected by chemiluminescence using Goat anti-Mouse IgG (H+L) Superclonal™ Secondary Antibody, HRP conjugate (Product # A28177, 0.25 µg/mL, 1:4000 dilution). A 102 kDa band corresponding to HK2 was observed across the cell lines tested.



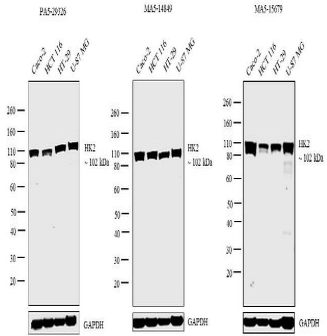
HK2 Antibody (MA5-15679) in IHC (P)

Immunohistochemical analysis of paraffin-embedded esophagus cancer tissues (left) and human lung cancer (right) using HK2 monoclonal antibody (Product # MA5-15679) followed with DAB staining.



HK2 Antibody (MA5-15679)

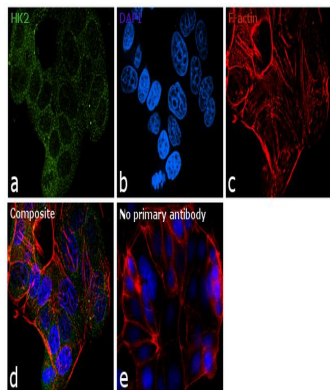
Antibody specificity was demonstrated by showing that antibodies raised against the same target protein perform similarly. Western blot of HK2 using HK2 Monoclonal Antibody (Product # MA5-15679), tested in parallel with HK2 Monoclonal Antibody (Product # MA5-14849) and HK2 Polyclonal Antibody (Product # PA5-29326), shows similar expression of HK2 in the cell lines tested. {IAV}



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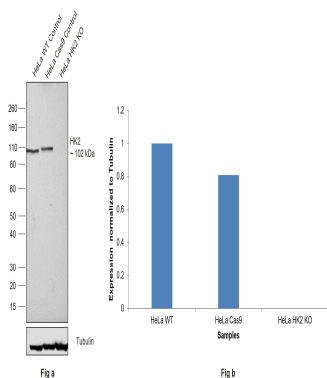


HK2 Antibody (MA5-15679) in ICC/IF

Immunofluorescence analysis of HK2 was performed using 70% confluent log phase MCF-7 cells. The cells were fixed with 4% paraformaldehyde for 10 minutes, permeabilized with 0.1% Triton™ X-100 for 15 minutes, and blocked with 1% BSA for 1 hour at room temperature. The cells were labeled with HK2 Monoclonal Antibody (3D3) (Product # MA5-15679) at 1:100 dilution in 0.1% BSA, incubated at 4 degree Celsius overnight and then labeled with Goat anti-Mouse IgG (Heavy Chain) Superclonal™ Secondary Antibody, Alexa Fluor® 488 conjugate (Product # A27034) at a dilution of 1:2000 for 45 minutes at room temperature (Panel a: green). Nuclei (Panel b: blue) were stained with SlowFade® Gold Antifade Mountant with DAPI (Product # S36938). F-actin (Panel c: red) was stained with Rhodamine Phalloidin (Product # R415, 1:300). Panel d represents the merged image showing cytoplasmic localization. Panel e represents control cells with no primary antibody to assess background. The images were captured at 60X magnification.

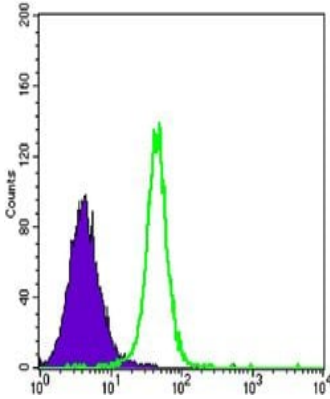
HK2 Antibody (MA5-15679) in WB

Knockout of HK2 was achieved by CRISPR-Cas9 genome editing using LentiArray™ Lentiviral sgRNA (Product # A32042, Assay ID CRISPR1034965_LV) and LentiArray Cas9 Lentivirus (Product # A32064). Western blot analysis of HK2 was performed by loading 30 µg of HeLa wild type (Lane 1), HeLa Cas9 (Lane 2) and HeLa HK2 KO (Lane 3) whole cell extracts. The samples were electrophoresed using NuPAGE™ Novex™ 4-12% Bis-Tris Protein Gel (Product # NP0322BOX). Resolved proteins were then transferred onto a nitrocellulose membrane (Product # IB23001) by iBlot® 2 Dry Blotting System (Product # IB21001). The blot was probed with HK2 Monoclonal Antibody (3D3) (Product # MA5-15679, 1:1000 dilution) and detected by Goat anti-Rabbit IgG (H+L) Superclonal™ Recombinant Secondary Antibody, HRP (Product # A27036, 1:10,000 dilution) using the iBright™ FL1500 (Product # A44115). Chemiluminescent detection was performed using SuperSignal™ West Dura Extended Duration Substrate (Product # 34076). Loss of signal upon CRISPR mediated knockout (KO) using the LentiArray™ CRISPR product line confirms that antibody is specific to HK2.



HK2 Antibody (MA5-15679) in Flow

Flow cytometric analysis of K562 cells using HK2 monoclonal antibody (Product # MA5-15679) (green) and negative control (purple).



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