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Anti-Hexokinase 1/HK1 Antibody Picoband™ (monoclonal, 2I4)

Catalog # ABO14939

Product Information

Application	WB, IHC, IHC-F, IF, ICC, FC
Primary Accession	P19367
Host	Mouse
Isotype	Mouse IgG2b
Reactivity	Rat, Human, Mouse
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-Hexokinase 1/HK1 Antibody Picoband™ (monoclonal, 2I4) . Tested in Flow Cytometry, IF, IHC, IHC-F, ICC, WB applications. This antibody reacts with Human, Mouse, Rat.
Reconstitution	Add 0.2ml of distilled water will yield a concentration of 500ug/ml.

Additional Information

Gene ID	3098
Other Names	Hexokinase-1, 2.7.1.1, Brain form hexokinase, Hexokinase type I, HK I, Hexokinase-A, HK1 (HGNC:4922)
Calculated MW	102486
Application Details	Western blot, 0.1-0.5 □g/ml, Human, Mouse, Rat Immunohistochemistry (Paraffin-embedded Section), 0.5-1 □g/ml, Human Immunohistochemistry (Frozen Section), 0.5-1 □g/ml, Human Immunocytochemistry/Immunofluorescence, 2 □g/ml, Human Immunofluorescence, 2 □g/ml, Human Flow Cytometry, 1-3 □g/1x10^6 cells, Human
Subcellular Localization	Cytosol. Mitochondrion outer membrane. Peripheral membrane protein.
Source	Eukaryota
Contents	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na_2HPO_4, 0.05mg NaN_3.
Clone Names	Clone: 2I4
Immunogen	E.coli-derived human Hexokinase 1/HK1 recombinant protein (Position: D17-R323).
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

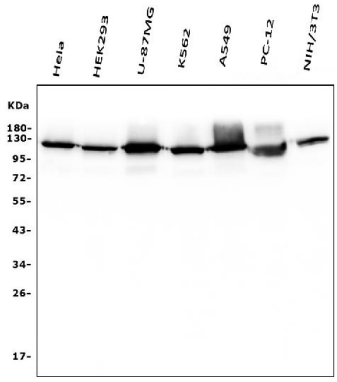
Name	HK1 (HGNC:4922)
Function	Catalyzes the phosphorylation of various hexoses, such as D- glucose, D-glucosamine, D-fructose, D-mannose and 2-deoxy-D-glucose, to hexose 6-phosphate (D-glucose 6-phosphate, D-glucosamine 6-phosphate, D-fructose 6-phosphate, D-mannose 6-phosphate and 2-deoxy-D-glucose 6- phosphate, respectively) (PubMed:1637300, PubMed:25316723, PubMed:27374331). Does not phosphorylate N-acetyl-D-glucosamine (PubMed:27374331). Mediates the initial step of glycolysis by catalyzing phosphorylation of D-glucose to D-glucose 6-phosphate (By similarity). Involved in innate immunity and inflammation by acting as a pattern recognition receptor for bacterial peptidoglycan (PubMed:27374331). When released in the cytosol, N-acetyl-D-glucosamine component of bacterial peptidoglycan inhibits the hexokinase activity of HK1 and causes its dissociation from mitochondrial outer membrane, thereby activating the NLRP3 inflammasome (PubMed:27374331).
Cellular Location	Mitochondrion outer membrane; Peripheral membrane protein. Cytoplasm, cytosol. Note=The mitochondrial-binding peptide (MBP) region promotes association with the mitochondrial outer membrane (Probable). Dissociates from the mitochondrial outer membrane following inhibition by N-acetyl-D-glucosamine, leading to relocation to the cytosol (PubMed:27374331).
Tissue Location	Isoform 2: Erythrocyte specific (Ref.6). Isoform 3: Testis-specific (PubMed:10978502). Isoform 4: Testis-specific (PubMed:10978502). {ECO:0000269|PubMed:10978502, ECO:0000269|Ref.6}

Background

Hexokinase-1 (HK1) is an enzyme that in humans is encoded by the HK1 gene on chromosome 10. It is mapped to 10q22.1. Hexokinases phosphorylate glucose to produce glucose-6-phosphate, the first step in most glucose metabolism pathways. This gene encodes a ubiquitous form of hexokinase which localizes to the outer membrane of mitochondria. Mutations in this gene have been associated with hemolytic anemia due to hexokinase deficiency. Alternative splicing of this gene results in several transcript variants which encode different isoforms, some of which are tissue-specific.

Images

Figure 1. Western blot analysis of Hexokinase 1/HK1 using anti-Hexokinase 1/HK1 antibody (M01504-1).
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 50ug of sample under reducing conditions.
Lane 1: human Hela whole cell lysates;
Lane 2: human HEK293 whole cell lysates;
Lane 3: human U-87MG whole cell lysates;



Lane 4: human K562 whole cell lysates;
Lane 5: human A549 whole cell lysates;
Lane 6: rat PC-12 whole cell lysates;
Lane 7: mouse NIH/3T3 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-Hexokinase 1/HK1 antigen affinity purified monoclonal antibody (Catalog # M01504-1) 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 Hexokinase 1/HK1 at approximately 120KD. The expected band size for Hexokinase 1/HK1 is at 120KD.

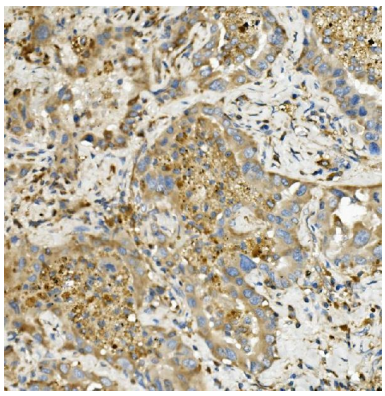


Figure 2. IHC analysis of Hexokinase 1/HK1 using anti-Hexokinase 1/HK1 antibody (M01504-1). Hexokinase 1/HK1 was detected in paraffin-embedded section of human lung cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-Hexokinase 1/HK1 Antibody (M01504-1) 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 Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

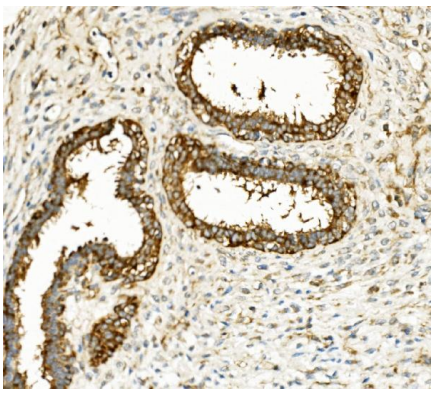


Figure 3. IHC analysis of Hexokinase 1/HK1 using anti-Hexokinase 1/HK1 antibody (M01504-1). Hexokinase 1/HK1 was detected in paraffin-embedded section of human mammary cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-Hexokinase 1/HK1 Antibody (M01504-1) 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 Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

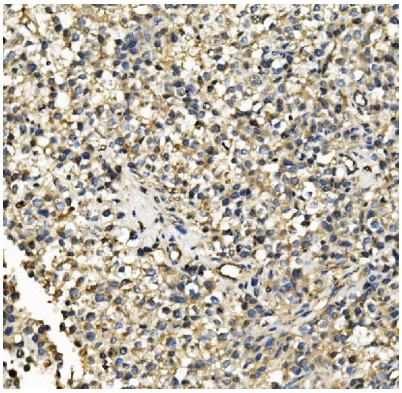


Figure 4. IHC analysis of Hexokinase 1/HK1 using anti-Hexokinase 1/HK1 antibody (M01504-1). Hexokinase 1/HK1 was detected in paraffin-embedded section of human testis cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-Hexokinase 1/HK1 Antibody (M01504-1) 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 Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

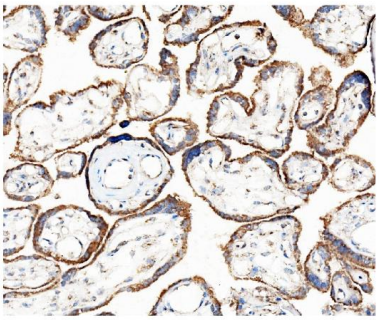


Figure 5. IHC analysis of Hexokinase 1/HK1 using anti-Hexokinase 1/HK1 antibody (M01504-1). Hexokinase 1/HK1 was detected in frozen section of human placenta tissue. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-Hexokinase 1/HK1 Antibody (M01504-1) 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 Strepavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

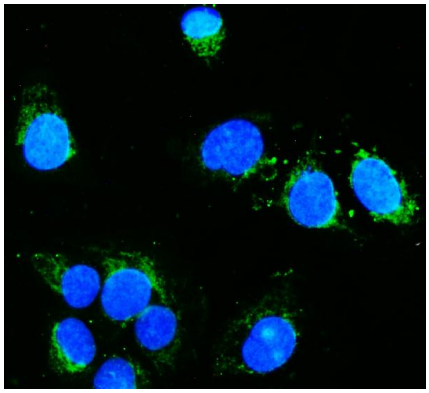


Figure 6. IF analysis of Hexokinase 1/HK1 using anti-Hexokinase 1/HK1 antibody (M01504-1). Hexokinase 1/HK1 was detected in immunocytochemical section of Hela cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 2 µg/mL mouse anti-Hexokinase 1/HK1 Antibody (M01504-1) 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.

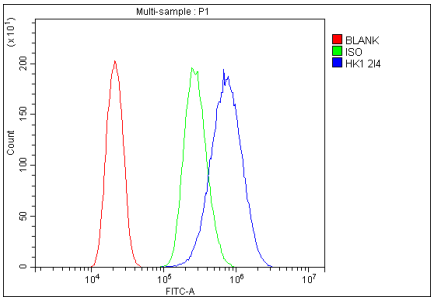


Figure 7. Flow Cytometry analysis of PC-3 cells using anti-Hexokinase 1/HK1 antibody (M01504-1). Overlay histogram showing PC-3 cells stained with M01504-1 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-Hexokinase 1/HK1 Antibody (M01504-1, 1 µg/1x10^6 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.

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