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Anti-Hexokinase 1/HK1 Antibody Picoband™ (monoclonal, 4B7)

Catalog # ABO14940

Specification

Anti-Hexokinase 1/HK1 Antibody Picoband™ (monoclonal, 4B7) - Product Information

Application	**WB, IHC, IHC-F, IF, ICC, FC**
Primary Accession	P19367
Host	**Mouse**
Isotype	**Mouse IgG2a**
Reactivity	**Rat, Human, Mouse**
Clonality	**Monoclonal**
Format	**Lyophilized**

Description
Anti-Hexokinase 1/HK1 Antibody Picoband™ (monoclonal, 4B7) . 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.

Anti-Hexokinase 1/HK1 Antibody Picoband™ (monoclonal, 4B7) - 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
120 kDa KDa

Application Details
Western blot, 0.1-0.5 µg/ml, Human, Mouse, Rat
 Immunohistochemistry (Paraffin-embedded Section), 0.5-1 µg/ml, Human, Mouse, Rat
 Immunohistochemistry (Frozen Section), 0.5-1 µg/ml, Human
 Immunofluorescence, 2 µg/ml, Human
 Immunocytochemistry/Immunofluorescence, 2 µg/ml, Human
 Flow Cytometry, 1-3 µg/1x10^6 cells, Human

Subcellular Localization
Cytosol. Mitochondrion outer membrane. Peripheral membrane protein.

Contents
Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na₂HPO₄, 0.05mg NaN₃.

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.

Anti-Hexokinase 1/HK1 Antibody Picoband™ (monoclonal, 4B7) - 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}

Anti-Hexokinase 1/HK1 Antibody Picoband™ (monoclonal, 4B7) - 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-Hexokinase 1/HK1 Antibody Picoband™ (monoclonal, 4B7) - Images

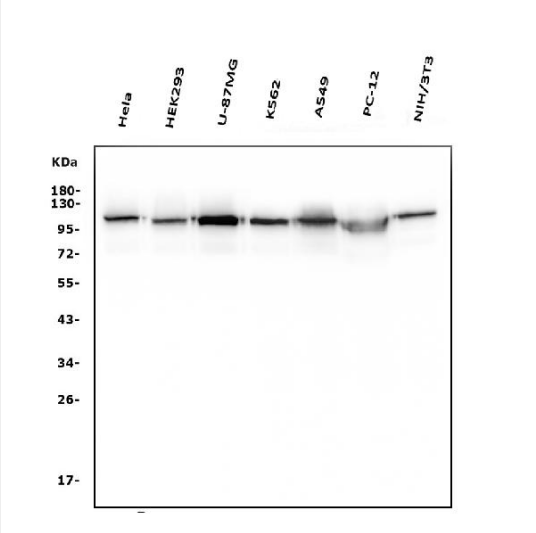

Figure 1. Western blot analysis of Hexokinase 1/HK1 using anti-Hexokinase 1/HK1 antibody (M01504-2).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.
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 # M01504-2) 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.

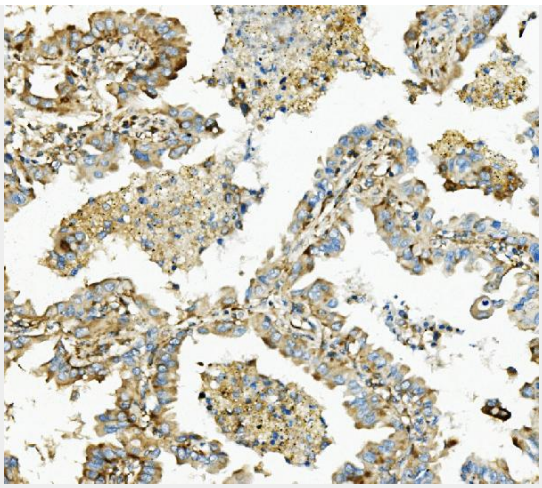

Figure 2. IHC analysis of Hexokinase 1/HK1 using anti-Hexokinase 1/HK1 antibody (M01504-2). Hexokinase 1/HK1 was detected in paraffin-embedded section of human lung cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was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

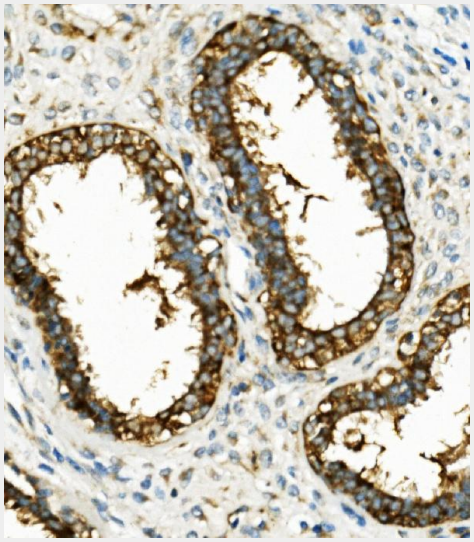

Figure 3. IHC analysis of Hexokinase 1/HK1 using anti-Hexokinase 1/HK1 antibody (M01504-2). Hexokinase 1/HK1 was detected in paraffin-embedded section of human mammary cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-Hexokinase 1/HK1 Antibody (M01504-2) 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.

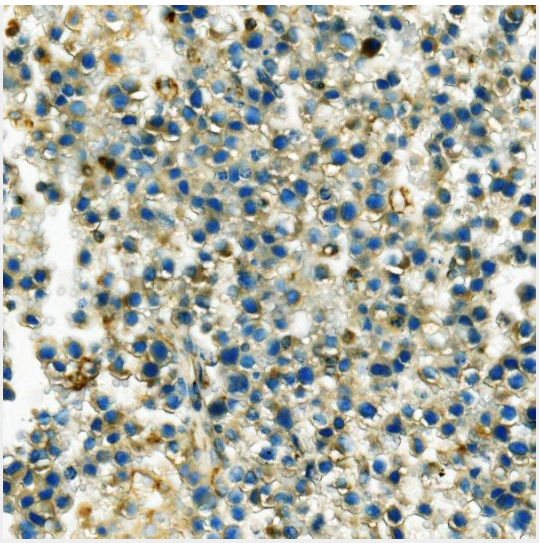

Figure 4. IHC analysis of Hexokinase 1/HK1 using anti-Hexokinase 1/HK1 antibody (M01504-2). Hexokinase 1/HK1 was detected in paraffin-embedded section of human testis cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-Hexokinase 1/HK1 Antibody (M01504-2) 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.

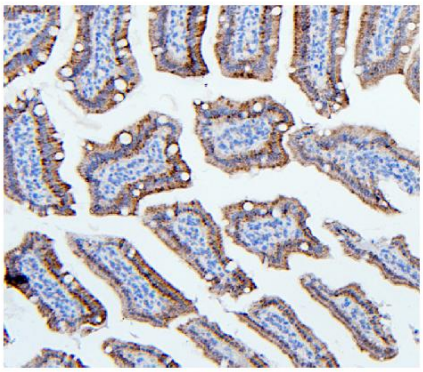

Figure 5. IHC analysis of Hexokinase 1/HK1 using anti-Hexokinase 1/HK1 antibody (M01504-2). Hexokinase 1/HK1 was detected in paraffin-embedded section of mouse intestine tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-Hexokinase 1/HK1 Antibody (M01504-2) 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.

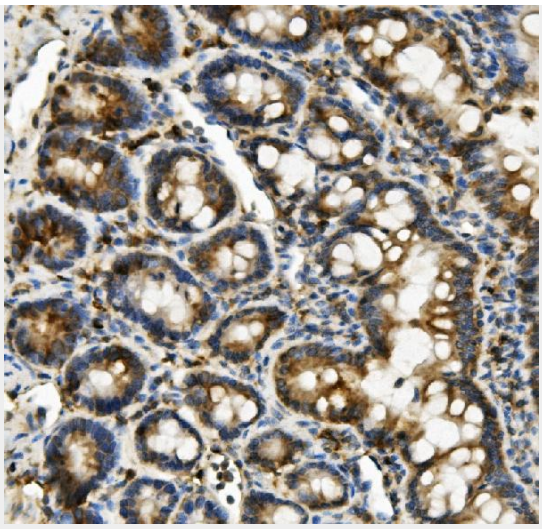

Figure 6. IHC analysis of Hexokinase 1/HK1 using anti-Hexokinase 1/HK1 antibody (M01504-2). Hexokinase 1/HK1 was detected in paraffin-embedded section of rat intestine tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-Hexokinase 1/HK1 Antibody (M01504-2) 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.

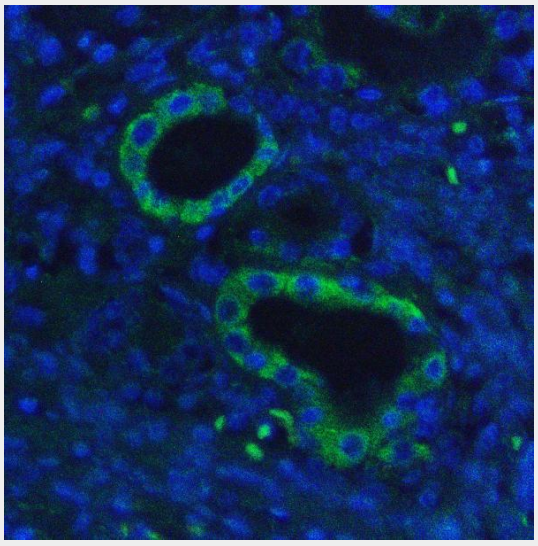

Figure 7. IF analysis of Hexokinase 1/HK1 using anti-Hexokinase 1/HK1 antibody (M01504-2). Hexokinase 1/HK1 was detected in paraffin-embedded section of human lung cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/mL mouse anti-Hexokinase 1/HK1 Antibody (M01504-2) 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.

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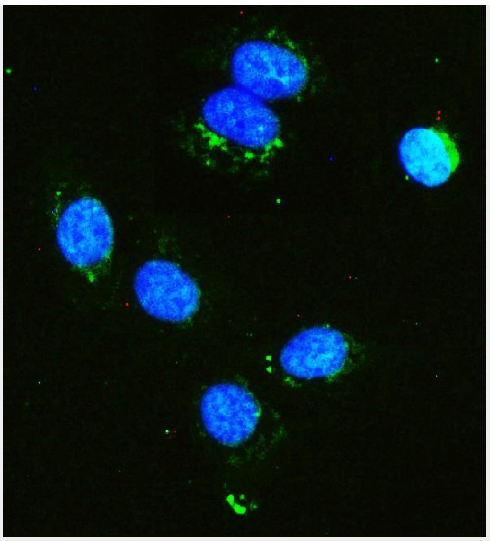

Figure 8. IF analysis of Hexokinase 1/HK1 using anti-Hexokinase 1/HK1 antibody (M01504-2). Hexokinase 1/HK1 was detected in immunocytochemical section of U20S cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 2 µg/mL mouse anti-Hexokinase 1/HK1 Antibody (M01504-2) 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.

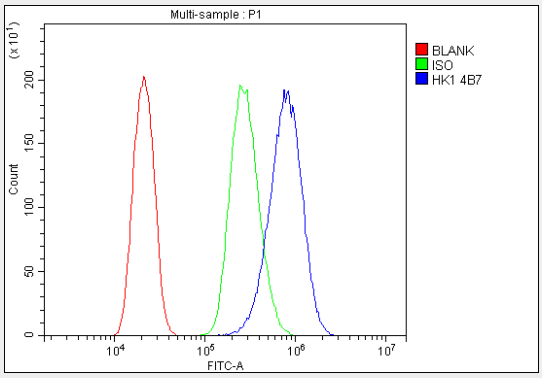

Figure 9. Flow Cytometry analysis of PC-3 cells using anti-Hexokinase 1/HK1 antibody (M01504-2).
Overlay histogram showing PC-3 cells stained with M01504-2 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-Hexokinase 1/HK1 Antibody (M01504-2, 1 µg/1x10^6 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.

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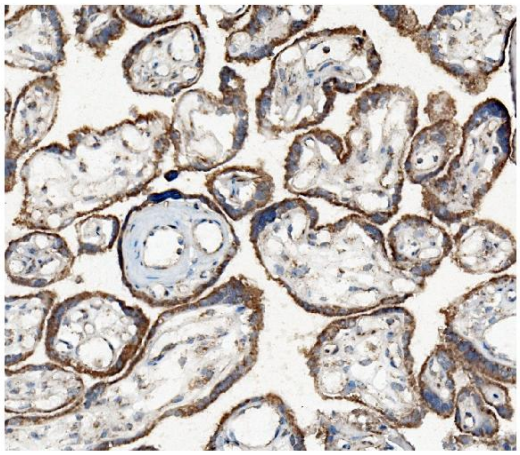

Figure 10. IHC analysis of Hexokinase 1/HK1 using anti-Hexokinase 1/HK1 antibody (M01504-2). Hexokinase 1/HK1 was detected in a frozen section of human placenta tissue. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-Hexokinase 1/HK1 Antibody (M01504-2) 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.

Anti-Hexokinase 1/HK1 Antibody Picoband™ (monoclonal, 4B7) - 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.