

## Product Datasheet

### Anti-Hexokinase 1/HK1 Antibody (monoclonal, 4B7) (orb654274)

<b>Description</b>	Anti-Hexokinase 1/HK1 Antibody (monoclonal, 4B7). Tested in Flow Cytometry, IF, IHC, IHC-F, ICC, WB applications. This antibody reacts with Human, Mouse, Rat.
<b>Species/Host</b>	Mouse
<b>Reactivity</b>	Human, Mouse, Rat
<b>Conjugation</b>	Unconjugated
<b>Tested Applications</b>	FC, ICC, IF, IHC, IHC-Fr, WB
<b>Immunogen</b>	E.coli-derived human Hexokinase 1/HK1 recombinant protein (Position: D17-R323).
<b>Form/Appearance</b>	Lyophilized
<b>Concentration</b>	Adding 0.2 ml of distilled water will yield a concentration of 500 µg/ml.
<b>Storage</b>	Maintain refrigerated at 2-8°C for up to 2 weeks. For long term storage store at -20°C in small aliquots to prevent freeze-thaw cycles.
<b>Note</b>	For research use only
<b>Application notes</b>	Western blot, 0.1-0.5µg/ml, Human, Mouse, Rat Immunohistochemistry (Paraffin-embedded Section), 0.5-1µg/ml, Human, Mouse, Rat Immunohistochemistry (Frozen Section), 0.5-1µg/ml, Human Immunofluorescence, 2µg/ml, Human Immunocytochemistry/Immunofluorescence, 2µg/ml, Human Flow Cytometry (Fixed), 1-3µg/1x10 <sup>6</sup> cells, Human. Add 0.2ml of distilled water will yield a concentration of 500ug/ml
<b>Isotype</b>	Mouse IgG2a
<b>Clonality</b>	Monoclonal

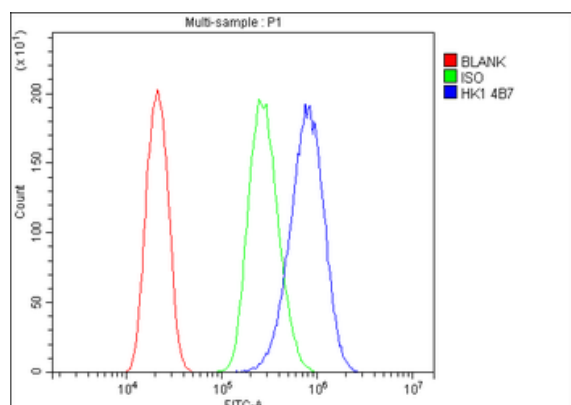
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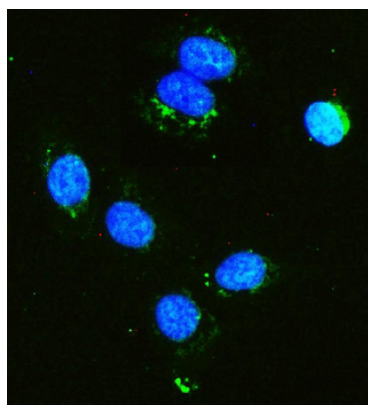
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<b>Clone Number</b>	4B7
<b>Antibody Type</b>	Primary Antibody
<b>MW</b>	120 kDa
<b>Uniprot ID</b>	<b>P19367</b>
<b>Expiration Date</b>	12 months from date of receipt.



Flow Cytometry analysis of PC-3 cells using anti-Hexokinase 1/HK1 antibody. Overlay histogram showing PC-3 cells (Blue line). To facilitate intracellular staining, cells were fixed with 4% paraformaldehyde and permeabilized with permeabilization buffer. The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-Hexokinase 1/HK1 Antibody (1  $\mu\text{g}/1 \times 10^6$  cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (5-10  $\mu\text{g}/1 \times 10^6$  cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1  $\mu\text{g}/1 \times 10^6$ ) used under the same conditions. Unlabelled sample without incubation with primary antibody and secondary antibody (Red line) was used as a blank control.



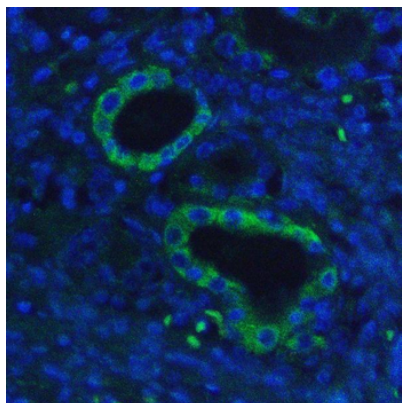
IF analysis of Hexokinase 1/HK1 using anti-Hexokinase 1/HK1 antibody. Hexokinase 1/HK1 was detected in immunocytochemical section of U20S cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 2  $\mu\text{g}/\text{mL}$  mouse anti-Hexokinase 1/HK1 Antibody overnight at 4°C. DyLight®488 Conjugated Goat Anti-Mouse IgG 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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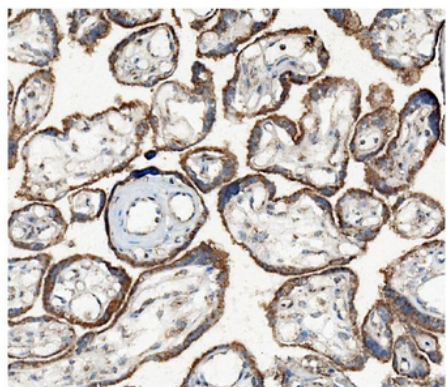
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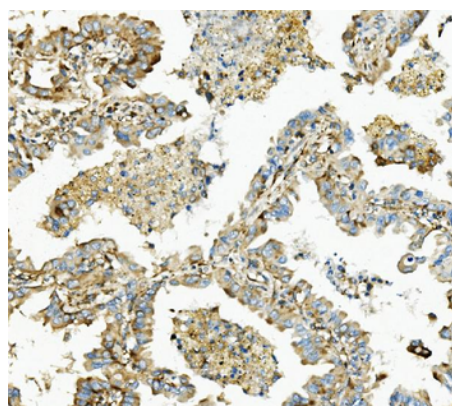
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IF analysis of Hexokinase 1/HK1 using anti-Hexokinase 1/HK1 antibody. Hexokinase 1/HK1 was detected in paraffin-embedded section of human lung cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). 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 overnight at 4°C. DyLight®488 Conjugated Goat Anti-Mouse IgG 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.



IHC analysis of Hexokinase 1/HK1 using anti-Hexokinase 1/HK1 antibody. 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 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) with DAB as the chromogen.



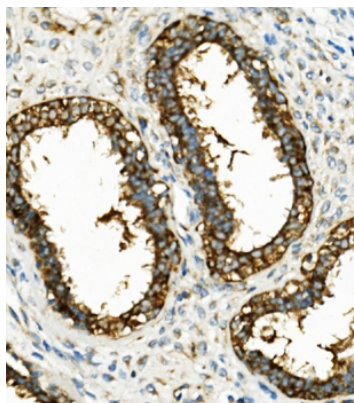
IHC analysis of Hexokinase 1/HK1 using anti-Hexokinase 1/HK1 antibody. Hexokinase 1/HK1 was detected in paraffin-embedded section of human lung cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat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

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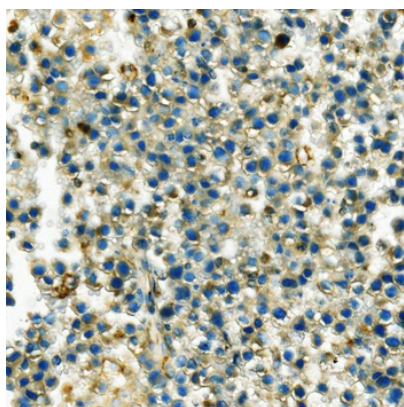
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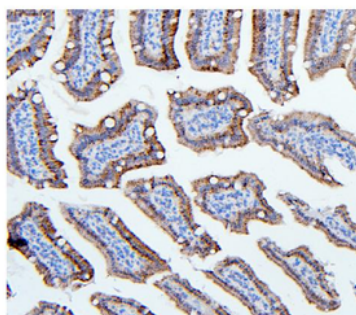
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IHC analysis of Hexokinase 1/HK1 using anti-Hexokinase 1/HK1 antibody. Hexokinase 1/HK1 was detected in paraffin-embedded section of human mammary cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval 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 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) with DAB as the chromogen.



IHC analysis of Hexokinase 1/HK1 using anti-Hexokinase 1/HK1 antibody. 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 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) with DAB as the chromogen.



IHC analysis of Hexokinase 1/HK1 using anti-Hexokinase 1/HK1 antibody. 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 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) with DAB as the chromogen.

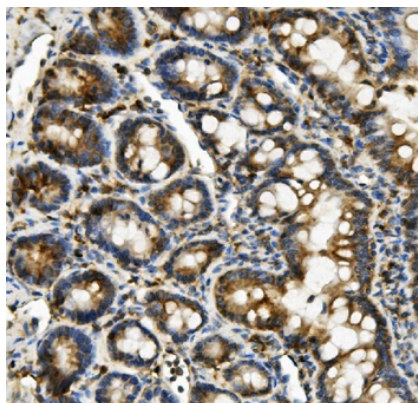
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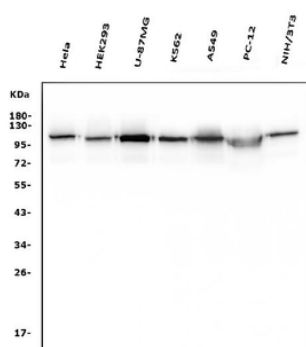
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IHC analysis of Hexokinase 1/HK1 using anti-Hexokinase 1/HK1 antibody. Hexokinase 1/HK1 was detected in paraffin-embedded section of rat intestine tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). 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 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) with DAB as the chromogen.



Western blot analysis of Hexokinase 1/HK1 using anti-Hexokinase 1/HK1 antibody. 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 50 µg 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 150 mA 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 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 with Tanon 5200 system. A specific band was detected for Hexokinase 1/HK1 at approximately 120 KD. The expected band size for Hexokinase 1/HK1 is at 120 KD.

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