# **Anti-Hexokinase 1 Antibody [ST47-05]**

### ET1609-28



Product Type: Recombinant Rabbit monoclonal IgG, primary antibodies

Species reactivity: Human, Mouse, Rat

Applications: WB, IF-Cell, IF-Tissue, IHC-P, IP, FC

Molecular Wt: Predicted band size: 102 kDa

Clone number: ST47-05

Description: The hexokinases utilize Mg-ATP as a phosphoryl donor to catalyze the first step of

intracellular glucose metabolism, the conversion of glucose to glucose-6-phosphate. Four hexokinase isoenzymes have been identified, including hexokinase I (HXK I), hexokinase II (HXK II), hexokinase III (HXK III) and hexokinase IV (HXK IV, also designated glucokinase or GCK). Hexokinases I-III each contain an N-terminal cluster of hydrophobic amino acids. Glucokinase lacks the N-terminal hydrophobic cluster. The hydrophobic cluster is thought to be necessary for membrane binding. This is substantiated by the finding that glucokinase has lower affinity for glucose than do the other hexokinases. HXK I has been shown to be expressed in brain, kidney and heart tissues as well as in hepatoma cell lines. HXK II is involved in the uptake and utilization of glucose by adipose and skeletal tissues. Of the hexokinases, HXK III has the highest affinity for glucose. Glucokinase is expressed in pancreatic beta cells where it functions as a glucose sensor, determining the "set point" for

insulin secretion.

**Immunogen:** Synthetic peptide within human Hexokinase 1 aa 100-140.

Positive control: Hela cell lysate, 293 cell lysate, MCF-7 cell lysate, HepG2 cell lysate, Hela, CRC, MCF-7,

human lung tissue, human breast carcinoma tissue, human kidney tissue, mouse colon

tissue, mouse brain tissue, mouse skin tissue, K562.

**Subcellular location:** Mitochondrion outer membrane, Cytoplasm, Membrane, Mitochondrion.

Database links: SwissProt: P19367 Human | P17710 Mouse | P05708 Rat

Recommended Dilutions:

 WB
 1:500-1:2,000

 IF-Cell
 1:50-1:200

 IF-Tissue
 1:50-1:200

 IHC-P
 1:50-1:200

 FC
 1:50-1:100

IP Use at an assay dependent concentration.

Storage Buffer: 1\*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw

cycles.

**Purity:** Protein A affinity purified.

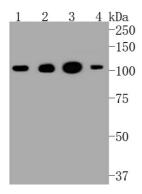
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Service mail:support@huabio.cn



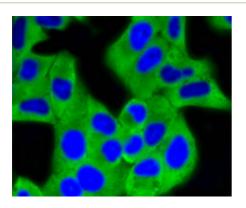
#### **Images**



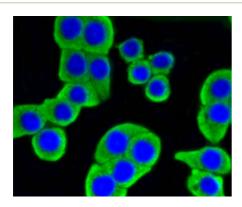
**Fig1:** Western blot analysis of Hexokinase 1 on different lysates. Proteins were transferred to a PVDF membrane and blocked with 5% BSA in PBS for 1 hour at room temperature. The primary antibody (ET1609-28, 1/500) was used in 5% BSA at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:5,000 dilution was used for 1 hour at room temperature.

#### Positive control:

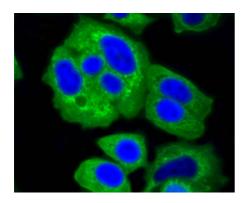
Lane 1: Hela cell lysate Lane 2: 293 cell lysate Lane 3: MCF-7 cell lysate Lane 4: HepG2 cell lysate



**Fig2:** ICC staining of Hexokinase 1 in Hela cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1609-28, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

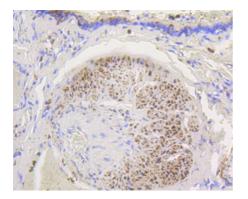


**Fig3:** ICC staining of Hexokinase 1 in CRC cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1609-28, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

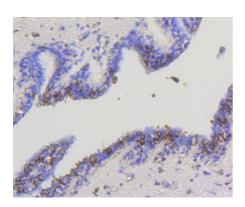


**Fig4:** ICC staining of Hexokinase 1 in MCF-7 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1609-28, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

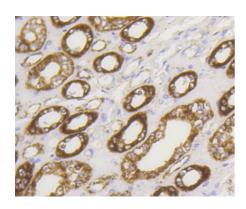
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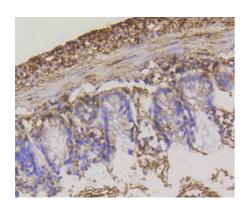
**Fig5:** Immunohistochemical analysis of paraffin-embedded human lung tissue using anti-Hexokinase 1 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (ET1609-28, 1/50) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.



**Fig6:** Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue using anti-Hexokinase 1 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (ET1609-28, 1/50) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.



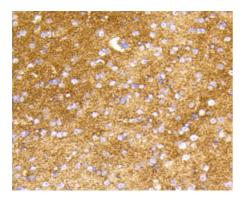
**Fig7:** Immunohistochemical analysis of paraffin-embedded human kidney tissue using anti-Hexokinase 1 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (ET1609-28, 1/50) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.



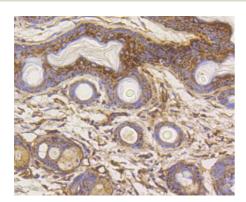
**Fig8:** Immunohistochemical analysis of paraffin-embedded mouse colon tissue using anti-Hexokinase 1 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH $_2$ O and PBS, and then probed with the primary antibody (ET1609-28, 1/50) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

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**Fig9:** Immunohistochemical analysis of paraffin-embedded mouse brain tissue using anti-Hexokinase 1 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (ET1609-28, 1/50) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.



**Fig10:** Immunohistochemical analysis of paraffin-embedded mouse skin tissue using anti-Hexokinase 1 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (ET1609-28, 1/50) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

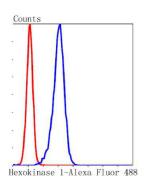


Fig11: Flow cytometric analysis of Hexokinase 1 was done on K562 cells. The cells were fixed, permeabilized and stained with the primary antibody (ET1609-28, 1/50) (blue). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor 488-conjugated Goat anti-Rabbit IgG Secondary antibody at 1/1000 dilution for 30 minutes. Unlabelled sample was used as a control (cells without incubation with primary antibody; red).

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

#### **Background References**

- 1. Vaca Jacome A.S., et al. N-terminome analysis of the human mitochondrial proteome. Proteomics 15:2519-2524(2015).
- 2. Burkard T.R., et al. Initial characterization of the human central proteome. BMC Syst. Biol. 5:17-17(2011).

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