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:	MCF7 cell lysate, NIH/3T3 cell lysate, C2C12 cell lysate, Hela cell lysate, 293 cell lysate, HepG2 cell lysate, C2C12, MCF7, human kidney tissue, mouse kidney tissue, rat kidney tissue.
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	1:100-1:500
IF-Tissue	1:200
IHC-P	1:1,000
FC	1:1,000
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:	Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.
Purity:	Protein A affinity purified.

Images

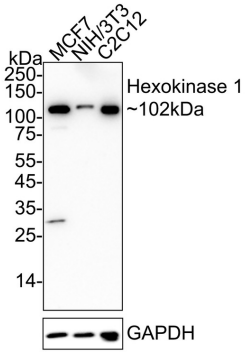

Fig1: Western blot analysis of Hexokinase 1 on different lysates with Rabbit anti-Hexokinase 1 antibody (ET1609-28) at 1/1,000 dilution.

Lane 1: MCF7 cell lysate
Lane 2: NIH/3T3 cell lysate
Lane 3: C2C12 cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 102 kDa
Observed band size: 102 kDa

Exposure time: 60 seconds;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1609-28) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

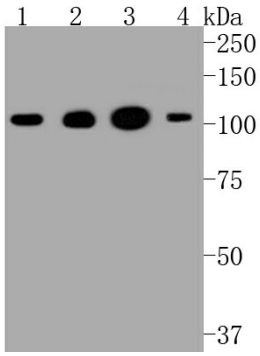

Fig2: Western blot analysis of Hexokinase 1 on different lysates. Proteins were transferred to a PVDF membrane and blocked with 5% BSA in PBS for 1 hour at room temperature. The primary antibody (ET1609-28, 1/500) was used in 5% BSA at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary 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: MCF7 cell lysate
Lane 4: HepG2 cell lysate

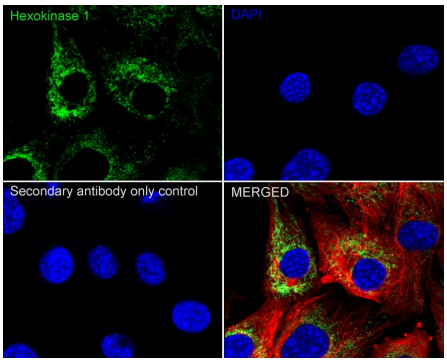

Fig3: Immunocytochemistry analysis of C2C12 cells labeling Hexokinase 1 with Rabbit anti-Hexokinase 1 antibody (ET1609-28) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-Hexokinase 1 antibody (ET1609-28) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

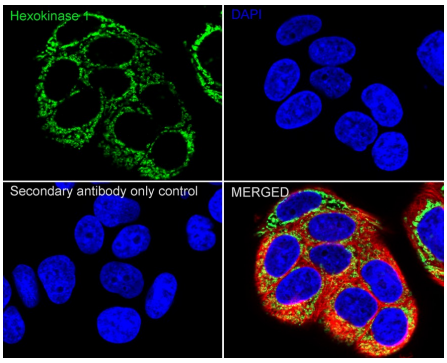

Fig4: Immunocytochemistry analysis of MCF7 cells labeling Hexokinase 1 with Rabbit anti-Hexokinase 1 antibody (ET1609-28) at 1/500 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-Hexokinase 1 antibody (ET1609-28) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

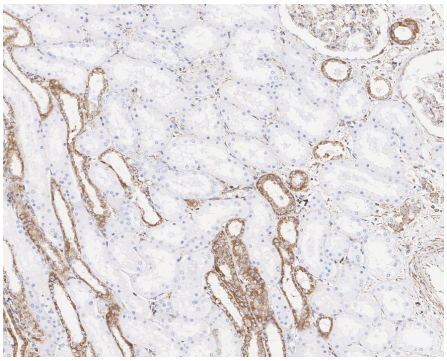

Fig5: Immunohistochemical analysis of paraffin-embedded human kidney tissue with Rabbit anti-Hexokinase 1 antibody (ET1609-28) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1609-28) at 1/1,000 dilution for 1 hour 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.

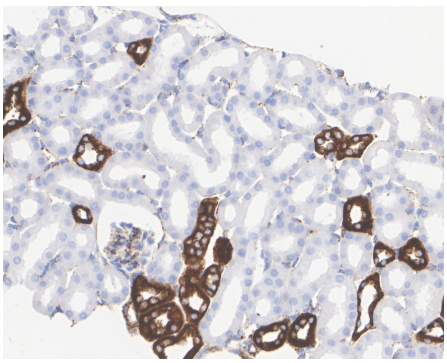

Fig6: Immunohistochemical analysis of paraffin-embedded mouse kidney tissue with Rabbit anti-Hexokinase 1 antibody (ET1609-28) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1609-28) at 1/1,000 dilution for 1 hour 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.

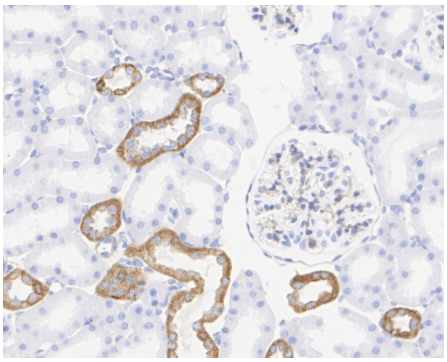

Fig7: Immunohistochemical analysis of paraffin-embedded rat kidney tissue with Rabbit anti-Hexokinase 1 antibody (ET1609-28) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1609-28) at 1/1,000 dilution for 1 hour 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.

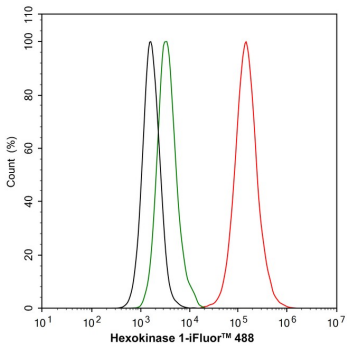

Fig8: Flow cytometric analysis of MCF7 cells labeling Hexokinase 1.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1609-28, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

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).