

Anti-Glucokinase Antibody [PSH28-07]

HA724749



Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Mouse, Rat
Applications:	WB, IHC-P, IF-Tissue, IP
Molecular Wt:	Predicted band size: 52 kDa
Clone number:	PSH28-07

Description: Glucokinase (EC 2.7.1.2) is an enzyme that facilitates phosphorylation of glucose to glucose-6-phosphate. Glucokinase occurs in cells in the liver and pancreas of humans and most other vertebrates. In each of these organs it plays an important role in the regulation of carbohydrate metabolism by acting as a glucose sensor, triggering shifts in metabolism or cell function in response to rising or falling levels of glucose, such as occur after a meal or when fasting. Mutations of the gene for this enzyme can cause unusual forms of diabetes or hypoglycemia. Glucokinase (GK) is a hexokinase isozyme, related homologously to at least three other hexokinases.[4] All of the hexokinases can mediate phosphorylation of glucose to glucose-6-phosphate (G6P), which is the first step of both glycogen synthesis and glycolysis. However, glucokinase is coded by a separate gene and its distinctive kinetic properties allow it to serve a different set of functions. Glucokinase has a lower affinity for glucose than the other hexokinases do, and its activity is localized to a few cell types, leaving the other three hexokinases as more important preparers of glucose for glycolysis and glycogen synthesis for most tissues and organs. Because of this reduced affinity, the activity of glucokinase, under usual physiological conditions, varies substantially according to the concentration of glucose.

Immunogen: Recombinant protein within mouse Glucokinase aa 1-465.

Positive control: Mouse liver tissue lysate, Rat liver tissue lysate.

Subcellular location: Cytoplasm, Nucleus, Mitochondrion.

Database links: SwissProt: P52792 Mouse | P17712 Rat

Recommended Dilutions:

WB	1:2,500
IHC-P	1:5,000
IF-Tissue	1:500
IP	1-2µg/sample

Storage Buffer: 1*PBS (pH7.4), 0.1% BSA, 40% Glycerol, 0.2% Proclean 950.

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). Store at -20℃ long term.

Purity: Protein A affinity purified.

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Orders:0086-571-88062880

Technical:0086-571-89986345

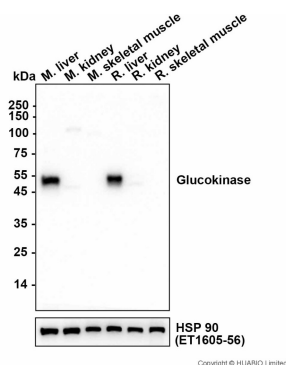
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Applications:WB=Western blot IHC-P=Immunohistochemistry (paraffin) IF-Cell=Immunofluorescence (Cell) IF-Tissue=Immunofluorescence (Tissue) FC=Flow cytometry IP=Immunoprecipitation

Images

Fig1: Western blot analysis of Glucokinase on different lysates with Rabbit anti-Glucokinase antibody (HA724749) at 1/2,500 dilution.



Lane 1: Mouse liver tissue lysate
 Lane 2: Mouse kidney tissue lysate
 Lane 3: Mouse skeletal muscle tissue lysate
 Lane 4: Rat liver tissue lysate
 Lane 5: Rat kidney tissue lysate
 Lane 6: Rat skeletal muscle tissue lysate

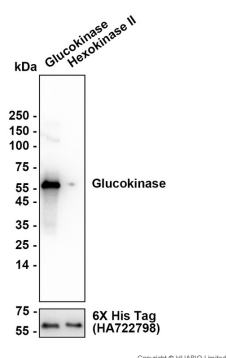
Lysates/proteins at 20 µg/Lane.
 Exposure time: 8 seconds; ECL: K1801

Low expression: skeletal muscle (PMID: 23085254).
 Low expression of Glucokinase protein in kidney is consistent with the predicted expression pattern.

Blocking: 5% NFDM/TBST, 1 hour at room temperature
 Primary antibody: HA724749, 1/2,500 in primary antibody dilution buffer (K1803), overnight at 4 °C
 Secondary antibody: Goat anti-Rabbit IgG-HRP (HA1001), 1/50,000 in 5% NFDM/TBST, 1 hour at room temperature

Predicted band size: 52 kDa
 Observed band size: 52 kDa

Fig2: Western blot analysis of Glucokinase on different lysates with Rabbit anti-Glucokinase antibody (HA724749) at 1/2,500 dilution.



Lane 1: His-tagged Human Glucokinase recombinant protein
 Lane 2: His-tagged Human Hexokinase II recombinant protein

Lysates/proteins at 10 ng/Lane.
 Exposure time: 4 seconds; ECL: K1801

Blocking: 5% NFDM/TBST, 1 hour at room temperature
 Primary antibody: HA724749, 1/2,500 in primary antibody dilution buffer (K1803), overnight at 4 °C
 Secondary antibody: Goat anti-Rabbit IgG-HRP (HA1001), 1/50,000 in 5% NFDM/TBST, 1 hour at room temperature

Predicted band size: 52 kDa
 Observed band size: 52 kDa

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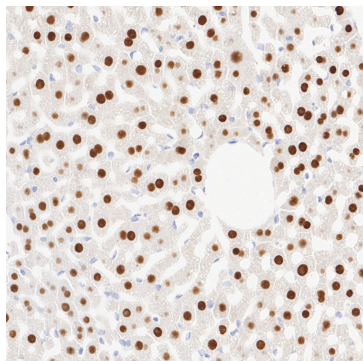
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Fig3: Application: Immunohistochemistry (IHC-P)

Species: Mouse

Tissue: Liver

Sample: Paraffin-embedded section



Antigen retrieval: Heat-mediated, Tris-EDTA buffer (pH 9.0), 20 minutes at 95°C.

Liver tissue from fasted mice. At low glucose concentrations, GCK binds to GCKR, and the resulting inactive complex is recruited into the hepatocyte nucleus (PMID: 24566088, PMID: 10713097).

Wash buffer: 1× TBST

Endogenous peroxidase blocking: 3% H₂O₂, 10 minutes at room temperature.

Blocking: 1% BSA + 10% normal goat serum, 10 minutes at room temperature.

Primary antibody: HA724749, 1/5,000, 1 hour at room temperature.

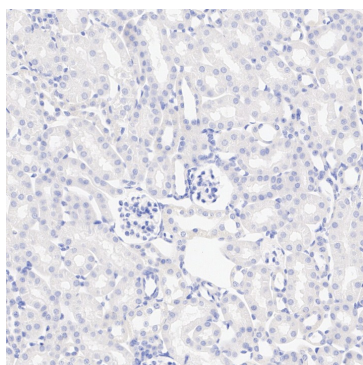
Secondary antibody: HA1119, 20 minutes at room temperature.

Fig4: Application: Immunohistochemistry (IHC-P)

Species: Mouse

Tissue: Kidney

Sample: Paraffin-embedded section



Antigen retrieval: Heat-mediated, Tris-EDTA buffer (pH 9.0), 20 minutes at 95°C.

Wash buffer: 1× TBST

Endogenous peroxidase blocking: 3% H₂O₂, 10 minutes at room temperature.

Blocking: 1% BSA + 10% normal goat serum, 10 minutes at room temperature.

Primary antibody: HA724749, 1/5,000, 1 hour at room temperature.

Secondary antibody: HA1119, 20 minutes at room temperature.

Low expression of Glucokinase protein in kidney is consistent with the predicted expression pattern.

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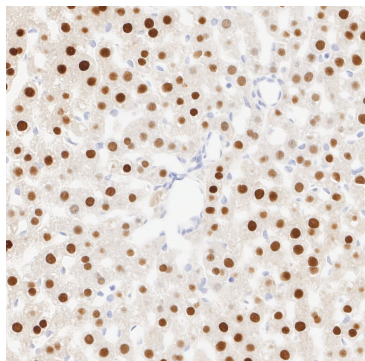
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Fig5: Application: Immunohistochemistry (IHC-P)

Species: Rat

Tissue: Liver

Sample: Paraffin-embedded section



Antigen retrieval: Heat-mediated, Tris-EDTA buffer (pH 9.0), 20 minutes at 95°C.

Liver tissue from fasted rats. At low glucose concentrations, GCK binds to GCKR, and the resulting inactive complex is recruited into the hepatocyte nucleus (PMID: 24566088, PMID: 10713097).

Wash buffer: 1× TBST

Endogenous peroxidase blocking: 3% H₂O₂, 10 minutes at room temperature.

Blocking: 1% BSA + 10% normal goat serum, 10 minutes at room temperature.

Primary antibody: HA724749, 1/5,000, 1 hour at room temperature.

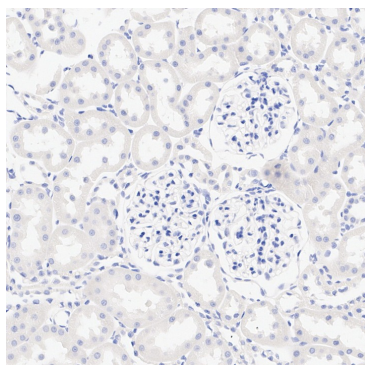
Secondary antibody: HA1119, 20 minutes at room temperature.

Fig6: Application: Immunohistochemistry (IHC-P)

Species: Rat

Tissue: Kidney

Sample: Paraffin-embedded section



Antigen retrieval: Heat-mediated, Tris-EDTA buffer (pH 9.0), 20 minutes at 95°C.

Wash buffer: 1× TBST

Endogenous peroxidase blocking: 3% H₂O₂, 10 minutes at room temperature.

Blocking: 1% BSA + 10% normal goat serum, 10 minutes at room temperature.

Primary antibody: HA724749, 1/5,000, 1 hour at room temperature.

Secondary antibody: HA1119, 20 minutes at room temperature.

Low expression of Glucokinase protein in kidney is consistent with the predicted expression pattern.

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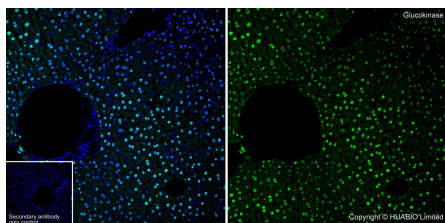
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Fig7: Application: Immunofluorescence (IF-tissue)

Species: Mouse

Tissue: Liver

Sample: Paraffin-embedded section



Antigen retrieval: Heat-mediated, Tris-EDTA buffer (pH 9.0), 20 minutes at 95°C.

Liver tissue from fasted mice. At low glucose concentrations, GSK binds to GSKR, and the resulting inactive complex is recruited into the hepatocyte nucleus (PMID: 24566088, PMID: 10713097).

Wash buffer: 1× TBST

Blocking: 10% normal goat serum + 1% Triton X-100 + 0.3 M Glycine in TBST, 30 minutes at room temperature.

Primary antibody: HA724749, 1/500, overnight at 4°C.

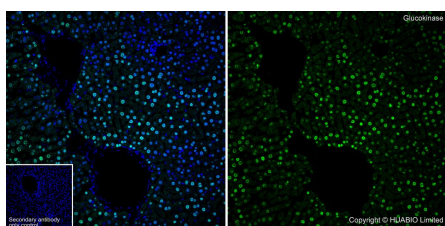
Secondary antibody: Goat Anti-Rabbit IgG (iFluor™ 488, HA1121), 1.5 hours at room temperature.

Fig8: Application: Immunofluorescence (IF-tissue)

Species: Rat

Tissue: Liver

Sample: Paraffin-embedded section



Antigen retrieval: Heat-mediated, Tris-EDTA buffer (pH 9.0), 20 minutes at 95°C.

Liver tissue from fasted rats. At low glucose concentrations, GSK binds to GSKR, and the resulting inactive complex is recruited into the hepatocyte nucleus (PMID: 24566088, PMID: 10713097).

Wash buffer: 1× TBST

Blocking: 10% normal goat serum + 1% Triton X-100 + 0.3 M Glycine in TBST, 30 minutes at room temperature.

Primary antibody: HA724749, 1/500, overnight at 4°C.

Secondary antibody: Goat Anti-Rabbit IgG (iFluor™ 488, HA1121), 1.5 hours at room temperature.

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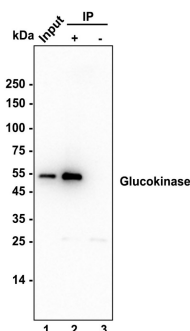
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Fig9: Immunoprecipitation (IP)

Glucokinase was immunoprecipitated in 0.2 mg Mouse liver tissue lysate with HA724749 at 2 µg/10 µl beads. Western blot was performed from the immunoprecipitate using HA724749 at 1/2,500 dilution. Anti-Rabbit IgG for IP, AlpSdAbs® VHH(HRP) (025-100-005) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: Mouse liver tissue lysate (input)

Lane 2: HA724749 IP in mouse tissue cell lysate

Lane 3: Rabbit IgG instead of HA724749 in mouse liver tissue lysate

Exposure time: 10 seconds

Blocking: 5% NFDM/TBST, 1 hour at room temperature

Primary dilution: HA724749, 1/2,500 in primary antibody dilution buffer (K1803), 2 hours at room temperature

Predicted band size: 52 kDa

Observed band size: 52 kDa

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

Background References

1. Ashcroft FM et al. Glucokinase activity in diabetes: too much of a good thing? Trends Endocrinol Metab. 2023 Feb
2. Ren Y et al. Glucokinase as an emerging anti-diabetes target and recent progress in the development of its agonists. J Enzyme Inhib Med Chem. 2022 Dec

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