Glycolysis Antibody Sampler Kit II

HAK21101

Contains Product	Quantity	Applications	Species reactivity	MW(kDa)
Aldolase [ET1705-91]	20μl	WB,IHC-P,IF-Tissue	H,M,R,Z	39 kDa
ENO1 [ET1705-56]	20μl	WB,FC,IF-Cell,IF-Tissue,IP	H,M,R,Z	47 kDa
NSE [ET1610-96]	20μl	WB,IF-Cell,IHC-P	H,M,R	47 kDa
PDK1 [ET1704-66]	20μl	WB,IHC-P,IP,FC,IF-Cell,IF-Tissue	H,M,R	49 kDa
PFKFB2 [HA500200]	20μl	WB,IHC-P	H,M	58 kDa
PFKFB3 [ET1705-66]	20μl	WB,IF-Cell,IHC-P,FC,IP,IF-Tissue	H,M,R	60 kDa
PGAM1 [ET7109-13]	20μl	WB,IHC-P,FC	H,M,R	29 kDa
HRP-Goat anti-Rabbit IgG [HA1001]	100μl	WB,ELISA,IHC-P	Rab	

Description: The Glycolysis II Antibody Sampler Kit provides an economical means to investigate select enzymes involved in glycolysis. The kit contains enough primary antibody to perform two western blot experiments per primary antibody.

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℃. Avoid repeated freeze / thaw cycles.

Background

Glycolysis is the metabolic process by which glucose is converted to pyruvate in a sequence of enzymatic steps. Phosphofructokinase (PFK) catalyzes the phosphorylation of fructose-6-phosphate in glycolysis. The bifunctional 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFK-2/FBPase or PFKFB) catalyzes the synthesis and degradation of fructose 2,6-bisphosphate and regulates its steady-state level. Four different PFKFB isoforms (PFKFB1, PFKFB2, PFKFB3, and PFKFB4) have been identified. Aldolase (fructose bisphosphate aldolase) is a glycolytic enzyme that catalyzes the conversion of fructose 1, 6-bisphosphate to 3-phosphoglyceraldehyde.

Phosphoglycerate mutase (PGAM1) catalyzes the conversion of 3-phosphoglycerate to 2-phosphoglycerate during glycolysis. Enolase is an important glycolytic enzyme involved in the interconversion of 2-phosphoglycerate to phosphoenolpyruvate. Mammalian enolase exists as three subunits: enolase-1 (α-enolase), enolase-2 (γ-enolase) and enolase-3 (β-enolase) that can form both homo- and heterodimers. Pyruvate dehydrogenase kinase (PDHK) phosphorylates PDH and inactivates it, whereas dephosphorylation of PDH is carried out by pyruvate dehydrogenase phosphatase to generate the active form.

Database links: UniProt ID: P04075, P05064, P05065, P06733, P17182, P04764, P09104, P17183, P07323, Q15118, Q8BFP9, Q63065, O60825, P70265, Q16875, O35552, Q3U3S6, P18669, Q9DBJ1, P25113

Images

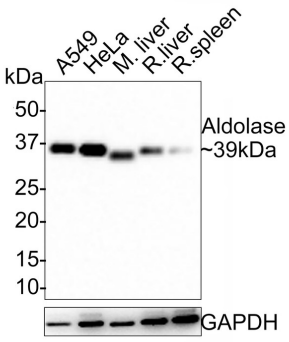

Fig1: Western blot analysis of Aldolase on different lysates with Rabbit anti-Aldolase antibody (ET1705-91) at 1/1,000 dilution.

Lane 1: A549 cell lysate (10 µg/Lane)
Lane 2: HeLa cell lysate (10 µg/Lane)
Lane 3: Mouse liver tissue lysate (20 µg/Lane)
Lane 4: Rat liver tissue lysate (20 µg/Lane)
Lane 5: Rat spleen tissue lysate (20 µg/Lane)

Predicted band size: 39 kDa
Observed band size: 39 kDa

Exposure time: 1 minute 20 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 (ET1705-91) at 1/1,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:100,000 dilution was used for 1 hour at room temperature.

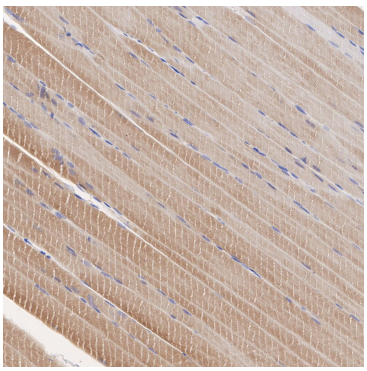

Fig2: Immunohistochemical analysis of paraffin-embedded rat skeletal muscle tissue with Rabbit anti-Aldolase antibody (ET1705-91) at 1/1,000 dilution.

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 (ET1705-91) 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.

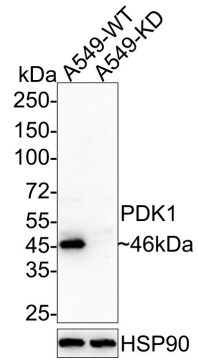

Fig3: Western blot analysis of PDK1 on different lysates with Rabbit anti-PDK1 antibody (ET1704-66) at 1/1,000 dilution.

Lane 1: A549-WT cell lysate
Lane 2: A549-KD PDK1 cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 49 kDa
Observed band size: 46 kDa

Exposure time: 3 minutes; ECL: K1801;

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 (ET1704-66) at 1/1,000 dilution was used in primary antibody diluent at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

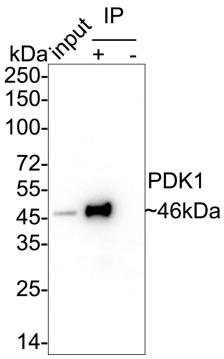

Fig4: PDK1 was immunoprecipitated from 0.2 mg mouse heart tissue lysate with ET1704-66 at 2 µg/25 µl agarose. Western blot was performed from the immunoprecipitate using ET1704-66 at 1/2,000 dilution. Anti-Rabbit IgG for IP Nano-secondary antibody (NBI01H) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: Mouse heart tissue lysate (input)
Lane 2: ET1704-66 IP in mouse heart tissue lysate
Lane 3: Rabbit IgG instead of ET1704-66 in mouse heart tissue lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 3 minutes; ECL: K1801

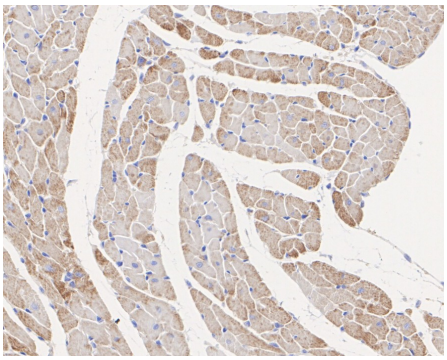

Fig5: Immunohistochemical analysis of paraffin-embedded rat heart tissue with Rabbit anti-PDK1 antibody (ET1704-66) at 1/2,000 dilution.

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 (ET1704-66) at 1/2,000 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.

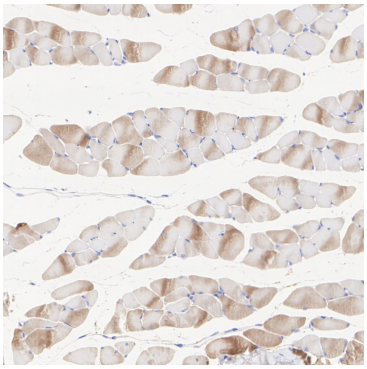

Fig6: Immunohistochemical analysis of paraffin-embedded mouse skeletal muscle tissue with Rabbit anti-Aldolase antibody (ET1705-91) at 1/1,000 dilution.

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 (ET1705-91) 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.

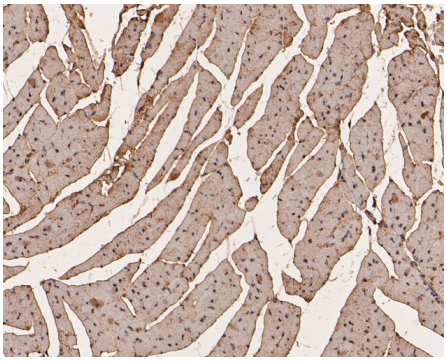

Fig7: Immunohistochemical analysis of paraffin-embedded mouse heart tissue using anti-PFKFB2 antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA500200, 1/400) 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.

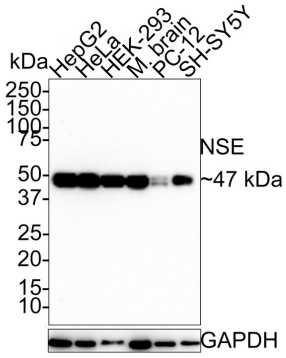

Fig8: Western blot analysis of NSE on different lysates with Rabbit anti-NSE antibody (ET1610-96) at 1/1,000 dilution.

Lane 1: HepG2 cell lysate (10 µg/Lane)
Lane 2: HeLa cell lysate (10 µg/Lane)
Lane 3: HEK-293 cell lysate (10 µg/Lane)
Lane 4: Mouse brain tissue lysate (20 µg/Lane)
Lane 5: PC-12 cell lysate (10 µg/Lane)
Lane 6: SH-SY5Y cell lysate (10 µg/Lane)

Predicted band size: 47 kDa
Observed band size: 47 kDa

Exposure time: 1 minutes;

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 (ET1610-96) at 1/1,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:100,000 dilution was used for 1 hour at room temperature.

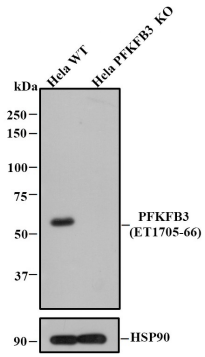

Fig9: All lanes: Western blot analysis of PFKFB3 with anti-PFKFB3 antibody [JM43-43] (ET1705-66) at 1/500 dilution.

Lane 1: Wild-type Hela whole cell lysate.
Lane 2: PFKFB3 knockout Hela whole cell lysate.

ET1705-66 was shown to specifically react with PFKFB3 in wild-type Hela cells. No band was observed when PFKFB3 knockout sample was tested. Wild-type and PFKFB3 knockout samples were subjected to SDS-PAGE. Proteins were transferred to a PVDF membrane and blocked with 5% NFDM in TBST for 1 hour at room temperature. The primary Anti-PFKFB3 antibody (ET1705-66, 1/500) and Anti-HSP90 antibody (ET1605-56, 1/10,000) were used in 5% BSA at room temperature for 2 hours. Goat Anti-Rabbit IgG H&L (HRP) Secondary Antibody (HA1001) at 1:200,000 dilution was used for 1 hour at room temperature.

Cell lysate was provided by Ubigene Biosciences (Ubigene Biosciences Co., Ltd., Guangzhou, China).

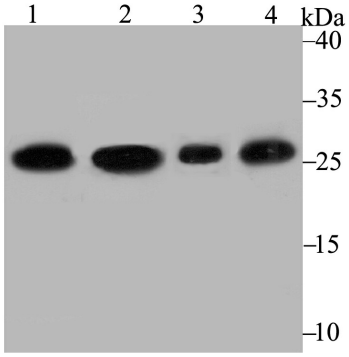

Fig10: Western blot analysis of PGAM1 on different lysates using anti-PGAM1 antibody at 1/2,000 dilution.
Positive control:
Lane 1: A431
Lane 2: A549
Lane 3: Rat brain
Lane 4: Mouse brain

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

Background References

1. Mediavilla D, Metón I, Baanante IV. Purification and kinetic characterization of 6-phosphofructo-1-kinase from the liver of gilthead sea bream (Sparus aurata). J Biochem. 2008 Aug;144(2):235-44.
2. Atsumi T, Nishio T, Niwa H, Takeuchi J, Bando H, Shimizu C, Yoshioka N, Bucala R, Koike T. Expression of inducible 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase/PFKFB3 isoforms in adipocytes and their potential role in glycolytic regulation. Diabetes. 2005 Dec;54(12):3349-57.
3. Wigfield SM, Winter SC, Giatromanolaki A, Taylor J, Koukourakis ML, Harris AL. PDK-1 regulates lactate production in hypoxia and is associated with poor prognosis in head and neck squamous cancer. Br J Cancer. 2008 Jun 17;98(12):1975-84.