Glycolysis/TCA Cycle Molecular Checkpoint Antibody Sampler Kit

HAK21054

Contains Product	Quantity	Applications	Species reactivity	MW(kDa)
PDHA1 [ET1702-75]	20µl	WB,IF-Cell,IF-Tissue,IHC-P,IP,FC	H,M,R	43 kDa
Phospho-PDHA1 (S293) [HA721443]	20µl	WB,ICC,IHC-P	H,M,R	43 kDa
PDK1 [ET1704-66]	20µl	WB,IHC-P,IP,FC,IF-Cell,IF-Tissue	H,M,R	49 kDa
LDHA [ER00702]	20µl	WB,IHC-P,FC,IF-Cell	H,M,R	37 kDa
LDHB [0807-1]	20µl	WB,IF-Cell,FC,IHC-P	H,M,R	37 kDa
HRP-Goat Anti-Rabbit IgG (H+L) [HA1001]	100ul	WB,ELISA,IHC-P	Rab	

Description: The Glycolysis/TCA Cycle Molecular Checkpoint Antibody Sampler Kit provides an economical means of detecting select components involved in the regulation of the connection between glycolysis and the citric acid cycle (tricarboxylic acid (TCA) cycle). The kit includes enough antibodies to perform two western blot experiments with each 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

The pyruvate dehydrogenase complex catalyzes the conversion of pyruvate and CoA into acetyl-CoA and CO2 in the presence of NAD+. Acetyl-CoA then goes into the citric acid cycle (tricarboxylic acid (TCA) cycle), where it reacts with oxaloacetate to form citrate.Pyruvate dehydrogenase kinase 1 (PDHK1) phosphorylates pyruvate dehydrogenase (E1) α1 subunit at Ser293 to inactivate its activity. This phosphorylation contributes to the tumor metabolic reprogramming toward glycolysis in hypoxia by inhibiting the citric acid cycle (TCA cycle).

Lactate dehydrogenase (LDH) catalyzes the reversible conversion between pyruvate and lactate. LDH is a tetramer composed of various combinations of LDHA subunit and LDHB subunit to form five different isozymes. LDHA has a higher affinity for pyruvate and preferentially catalyzes the conversion of pyruvate to lactate. In addition, acetylation of LDHB inhibits its activity, reduces hepatic lactate clearance, and promotes the progression of non-alcoholic fatty liver disease (NAFLD).

Database links: UniProt ID: P08559, P35486, P26284, P08559, P35486, P26284, Q15118, Q8BFP9, Q63065, P00338, P06151, P04642, P07195, P16125, P42123

Images

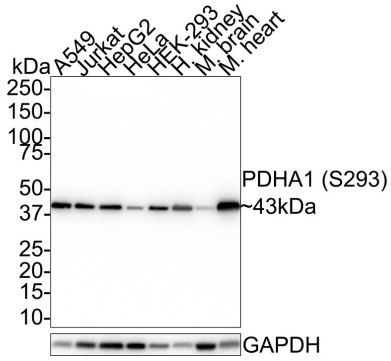

Fig1: Western blot analysis of Phospho-PDHA1 (S293) on different lysates with Rabbit anti-Phospho-PDHA1 (S293) antibody (HA721443) at 1/1,000 dilution.

Lane 1: A549 cell lysate (20 µg/Lane)
Lane 2: Jurkat cell lysate (20 µg/Lane)
Lane 3: HepG2 cell lysate (20 µg/Lane)
Lane 4: HeLa cell lysate (20 µg/Lane)
Lane 5: HEK-293 cell lysate (20 µg/Lane)
Lane 6: Human kidney tissue lysate (40 µg/Lane)
Lane 7: Mouse brain tissue lysate (40 µg/Lane)
Lane 8: Mouse heart tissue lysate (40 µg/Lane)

Predicted band size: 43 kDa
Observed band size: 43 kDa

Exposure time: 39 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 (HA721443) 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.

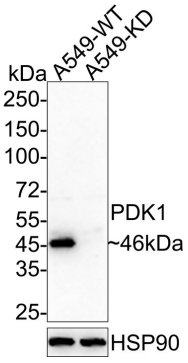

Fig2: Western blot analysis of PDK1 on different lysates with Rabbit anti-PDK1 antibody (ET1704-66) at 1/1,000 dilution.

Lane 1: A549-WT cell lysate (10 µg/Lane)
Lane 2: A549-KD PDK1 cell lysate (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.

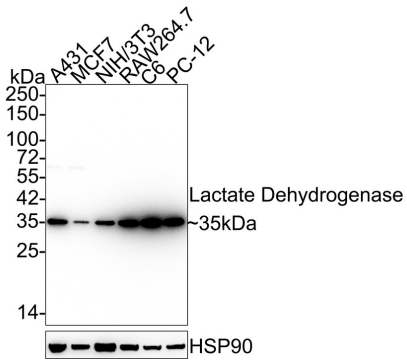

Fig3: Western blot analysis of LDHA on different lysates with Rabbit anti-LDHA antibody (ER00702) at 1/5,000 dilution.

Lane 1: A431 cell lysate (15 µg/Lane)
Lane 2: MCF7 cell lysate (15 µg/Lane)
Lane 3: NIH/3T3 cell lysate (15 µg/Lane)
Lane 4: RAW264.7 cell lysate (15 µg/Lane)
Lane 5: C6 cell lysate (15 µg/Lane)
Lane 6: PC-12 cell lysate (15 µg/Lane)

Predicted band size: 37 kDa
Observed band size: 35 kDa

Exposure time: 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 (ER00702) at 1/5,000 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.

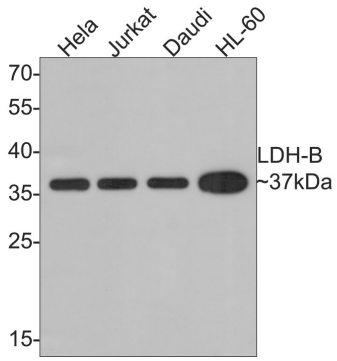

Fig4: Western blot analysis of LDHB on different lysates with Rabbit anti-LDHB antibody (0807-1) at 1/500 dilution.

Lane 1: Hela cell lysate (10 µg/Lane)
Lane 2: Jurkat cell lysate (10 µg/Lane)
Lane 3: Daudi cell lysate (10 µg/Lane)
Lane 4: HL-60 cell lysate (10 µg/Lane)

Predicted band size: 37 kDa
Observed band size: 37 kDa

Exposure time: 2 minutes;

12% 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 (0807-1) at 1/500 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:300,000 dilution was used for 1 hour at room temperature.

Background References

1. Hu Z, Yu X, Ding R, Liu B, Gu C, Pan XW, Han Q, Zhang Y, Wan J, Cui XG, Sun J, Zou Q. Glycolysis drives STING signaling to facilitate dendritic cell antitumor function. J Clin Invest. 2023 Apr 3;133(7):e166031.
2. Wang T, Chen K, Yao W, Zheng R, He Q, Xia J, Li J, Shao Y, Zhang L, Huang L, Qin L, Xu M, Zhang Z, Pan D, Li Z, Huang F. Acetylation of lactate dehydrogenase B drives NAFLD progression by impairing lactate clearance. J Hepatol. 2021 May;74(5):1038-1052.