Glycolysis Antibody Kit

HAK21008

Contains Product	Specification	Applications	Species reactivity	MW(kDa)
PKM2[R1603-5]	20μl	WB,IF-Cell,IHC-P,FC	H,M,R	58 kDa
GAPDH[ET1601-4]	20μl	WB,IF-Cell,IF-Tissue,IHC-P,FC,IP	H,M,R,C	36 kDa
PDHA1[ET1702-75]	20μl	WB,IF-Cell,IF-Tissue,IHC-P,IP,FC	H,M,R	43 kDa
Hexokinase I[ET1609-28]	20μl	WB,IF-Cell,IF-Tissue,IHC-P,IP,FC	H,M	102 kDa
Hexokinase II[HA500186]	20μl	WB,IF-Cell,IHC-P,FC	H,M,R	102 kDa
Lactate Dehydrogenase[ET1608-57]	20μl	WB,IF-Cell,IF-Tissue,IHC-P,FC,IP	H,M	37 kDa
PFKP[HA500472]	20μl	WB,IHC-P	H,M,R	86 kDa
HRP-Alpaca anti-Rabbit IgG Fc, Recombinant VHH[HA1031]	100μl	IP,ELISA,IHC-P,WB	Rab	

Description: The Glycolysis 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 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 Glycolysis is the metabolic process by which glucose is converted to pyruvate in a sequence of enzymatic steps. Hexokinase catalyzes the conversion of glucose to glucose-6-phosphate, the first step in glycolysis. Hexokinases I, II, and III are associated with the outer mitochondrial membrane and are critical for maintaining an elevated rate of aerobic glycolysis in cancer cells (Warburg effect). Phosphofructokinase (PFK) catalyzes the phosphorylation of fructose-6-phosphate. Platelet-type phosphofructokinase (PFKP) is expressed in various cell types. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) catalyzes the phosphorylation of glyceraldehyde-3-phosphate. Pyruvate kinase, a glycolytic enzyme, catalyses the conversion of phosphoenolpyruvate to pyruvate. In mammals, the M1 isoform (PKM1) is expressed in most adult tissues. The M2 isoform (PKM2), an alternatively-spliced variant of M1, is expressed during embryonic development. Lactate dehydrogenase (LDH) catalyzes the interconversion of pyruvate and NADH to lactate and NAD+. LDHA expression is induced when the oxygen supply is too low for mitochondrial ATP production. The pyruvate dehydrogenase complex catalyzes the conversion of pyruvate and CoA into acetyl-CoA and CO2 in the presence of NAD+. The reaction of oxidative decarboxylation of pyruvate serves as a critical link between glycolysis and the citric acid cycle and lipid metabolism.

Database links: UniProt ID: P14618, P52480, P11980, P04406, P16858, P04797, P08559, P35486, P26284, P19367, P17710, P05708, P52789, O08528, P27881, P07195, P00338, P16125, P06151, P42123, P04642, Q01813, Q9WUA3, P47860

Images

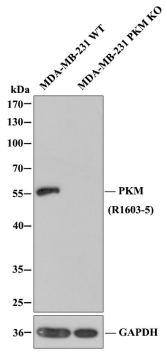

Fig1: All lanes: Western blot analysis of PKM with anti-PKM antibody (R1603-5) at 1:500 dilution.

Lane 1: Wild-type MDA-MB-231 whole cell lysate.
Lane 2: PKM knockout MDA-MB-231 whole cell lysate.

R1603-5 was shown to specifically react with PKM in wild-type MDA-MB-231 cells. No band was observed when PKM knockout sample was tested. Wild-type and PKM 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 antibody (R1603-5, 1/500) and Loading control antibody (Rabbit anti-GAPDH , ET1601-4, 1/10,000) was used in 5% BSA at room temperature for 2 hours. Goat Anti-Rabbit IgG-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).

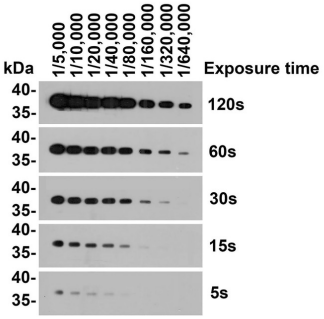

Fig2: Western blot analysis of GAPDH on Hela cell lysates with Rabbit anti-GAPDH antibody (ET1601-4).

Hela cell lysates at 10 µg/Lane.

Predicted band size: 36 kDa
Observed band size: 36 kDa

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 (ET1601-4) at serial 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.

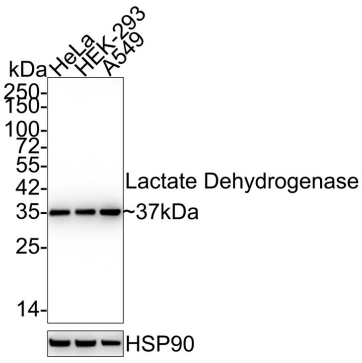

Fig3: Western blot analysis of Lactate Dehydrogenase on different lysates with Rabbit anti-Lactate Dehydrogenase antibody (ET1608-57) at 1/5,000 dilution.

Lane 1: HeLa cell lysate (15 µg/Lane)
Lane 2: HEK-293 cell lysate (15 µg/Lane)
Lane 3: A549 cell lysate (15 µg/Lane)

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

Exposure time: 1 minute 36 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 (ET1608-57) 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.

Background References

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3. Barber, R.D. et al. (2005) Physiol Genomics 21, 389-95.
4. Christofk, H.R. et al. (2008) Nature 452, 230-3.
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7. Strumiło, S. (2005) Acta Biochim Pol 52, 759-64.