abcam

Product datasheet

Anti-ketohexokinase antibody [EPR15847] ab197593





1 References 7 Images

Overview

Product name Anti-ketohexokinase antibody [EPR15847]

Rabbit monoclonal [EPR15847] to ketohexokinase **Description**

Host species Rabbit

Tested applications Suitable for: WB, IHC-P, ICC/IF, IP Species reactivity Reacts with: Mouse, Rat, Human

Immunogen Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: HEK293T cell lysate; Human fetal kidney and fetal liver lysates; Mouse kidney, mouse liver,

rat kidney and rat liver lysates. IHC-P: Human liver tissue. ICC/IF: HepG2 cells. IP: Human fetal

kidney lysate.

General notes This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility

- Improved sensitivity and specificity - Long-term security of supply - Animal-free production

For more information see here.

Our $\mathsf{RabMAb}^{\mathsf{®}}$ technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**® **patents**.

Properties

Form Liquid

Storage instructions Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long

term. Avoid freeze / thaw cycle.

Storage buffer pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol, 0.05% BSA

Purity Protein A purified

Clonality Monoclonal Clone number EPR15847

Isotype IgG

Applications

The Abpromise guarantee

Our <u>Abpromise guarantee</u> covers the use of ab197593 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		1/2500. Detects a band of approximately 33 kDa (predicted molecular weight: 33 kDa).
IHC-P		1/500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		1/1000.
IP		1/100.

Target

Tissue specificity Most abundant in liver, kidney, gut, spleen and pancreas. Low levels also found in adrenal,

muscle, brain and eye.

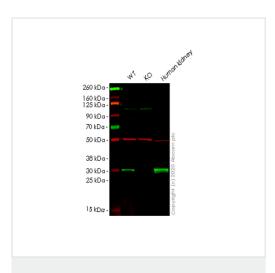
Pathway Carbohydrate metabolism; fructose metabolism.

Involvement in disease Defects in KHK are the cause of fructosuria (FRUCT) [MIM:229800]. Fructosuria is a benign

defect of intermediary metabolism.

Sequence similarities Belongs to the carbohydrate kinase pfkB family.

Images



Western blot - Anti-ketohexokinase antibody [EPR15847] (ab197593)

All lanes : Anti-ketohexokinase antibody [EPR15847] (ab197593) at 1/1000 dilution

Lane 1: Wild-type HEK293T cell lysate

Lane 2: KHK knockout HEK293T cell lysate

Lane 3: Human kidney tissue lysate

Lysates/proteins at 20 µg per lane.

Secondary

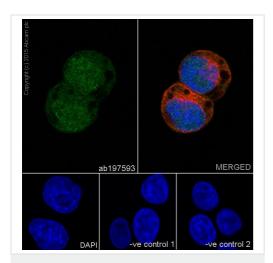
All lanes: Goat anti-Rabbit lgG H&L (IRDye® 800CW)

preadsorbed (ab216773) at 1/10000 dilution

Predicted band size: 33 kDa **Observed band size:** 33 kDa

Lanes 1-3: Merged signal (red and green). Green - ab197593 observed at 33 kDa. Red - loading control <u>ab7291</u> observed at 50 kDa.

ab197593 Anti-ketohexokinase antibody [EPR15847] was shown to specifically react with ketohexokinase in wild-type HEK293T cells. Loss of signal was observed when knockout cell line ab267292 (knockout cell lysate ab258017) was used. Wild-type and ketohexokinase knockout samples were subjected to SDS-PAGE. ab197593 and Anti-alpha Tubulin antibody [DM1A] - Loading Control (ab7291) were incubated overnight at 4°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit lgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse lgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

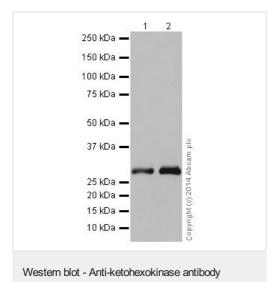


Immunocytochemistry/ Immunofluorescence - Antiketohexokinase antibody [EPR15847] (ab197593)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HepG2 (Human liver hepatocellular carcinoma) cells labeling ketohexokinase with ab197593 at 1/1000 dilution, followed by Goat anti-rabbit lgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/500 dilution (green). Cytoplasm and nuclear staining on HepG2 cell line is observed. The nuclear counter stain is DAPI (blue). Tubulin is detected with ab7291 (anti-Tubulin mouse mAb) at 1/1000 dilution and ab150120 (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution (red).

The negative controls are as follows:

-ve control 1: ab197593 at 1/1000 dilution followed by <u>ab150120</u> (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution. -ve control 2: <u>ab7291</u> (anti-Tubulin mouse mAb) at 1/1000 dilution followed by <u>ab150077</u> (Alexa Fluor®488 Goat Anti-Rabbit lgG H&L) at 1/500 dilution.



[EPR15847] (ab197593)

All lanes : Anti-ketohexokinase antibody [EPR15847] (ab197593) at 1/5000 dilution

Lane 1 : Human fetal kidney lysate

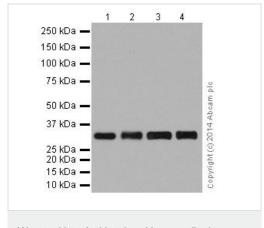
Lane 2 : Human fetal liver lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

Predicted band size: 33 kDa Observed band size: 33 kDa



Western blot - Anti-ketohexokinase antibody [EPR15847] (ab197593) Blocking/Dilution buffer: 5% NFDM/TBST.

All lanes : Anti-ketohexokinase antibody [EPR15847] (ab197593) at 1/2500 dilution

Lane 1 : Mouse kidney lysate
Lane 2 : Mouse liver lysate
Lane 3 : Rat kidney lysate
Lane 4 : Rat liver lysate

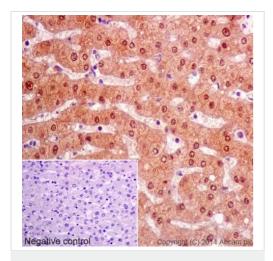
Lysates/proteins at 10 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugated at 1/1000 dilution

Predicted band size: 33 kDa **Observed band size:** 33 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

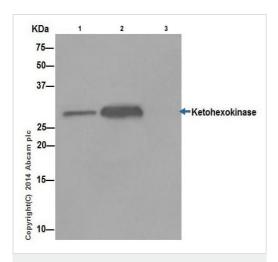


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-ketohexokinase antibody [EPR15847] (ab197593)

Immunohistochemical analysis of paraffin-embedded Human liver tissue labeling ketohexokinase with ab197593 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) secondary antibody at 1/500 dilution. Cytoplasm and nuclear staining on Human liver tissues is observed. Counter stained with Hematoxylin.

Negative control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

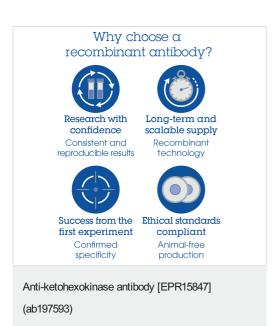


Immunoprecipitation - Anti-ketohexokinase antibody [EPR15847] (ab197593)

Ketohexokinase was immunoprecipitated from Human fetal kidney lysate with ab197593 at 1/100 dilution. Western blot was performed from the immunoprecipitate using ab197593 at 1/2000 dilution. Anti-Rabbit lgG (HRP), specific to the non-reduced form of lgG, was used as secondary antibody at 1/1500 dilution.

Lane 1: Human fetal kidney lysate 10 µg (Input). Lane 2: ab197593 IP in Human fetal kidney lysate. Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab197593 in Human fetal kidney lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.



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