# abcam

## Product datasheet

## Anti-Hexokinase 1 antibody [EPR10134(B)] - Mitochondrial Outer Membrane Marker ab150423





重组 RabMAb

★★★★★ 4 Abreviews 12 References 10 图像

#### 概述

产品名称 Anti-Hexokinase 1抗体[EPR10134(B)] - Mitochondrial Outer膜Marker

描述 兔单克隆抗体[EPR10134(B)] to Hexokinase 1 - Mitochondrial Outer膜Marker

宿主 Rabbit

经测试应用 适用于: WB, IHC-P, Flow Cyt (Intra), ICC/IF, mIHC

种属反应性 与反应: Mouse. Rat. Human

免疫原 Synthetic peptide corresponding to Human Hexokinase 1 aa 100-200 (internal sequence).

阳性对照 WB: HEK-293T and MCF7 cells, human mouse and rat brain lysates; IHC: Human, mouse and rat

kidney tissue; ICC/IF: MCF7 lysate; Flow Cyt (intra): K-562 cells. mIHC-P: Human kidney tissue.

常规说明 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 RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

#### 性能

形式 Liquid

存放说明 Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C.

Avoid freeze / thaw cycle.

pH: 7.20 存储溶液

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

纯度 Protein A purified

克隆 单克隆

克隆编号 EPR10134(B)

**同种型** IgG

#### 应用

The Abpromise guarantee Abpromise™承诺保证使用ab150423于以下的经测试应用

"应用说明"部分下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
WB	<b>★★★★★ (4)</b>	1/1000 - 1/10000. Predicted molecular weight: 102 kDa.
IHC-P		1/50 - 1/100. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
Flow Cyt (Intra)		1/20.  ab172730 - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
ICC/IF		1/50.
mIHC		1/250.

### 靶标

组织**特异性** Isoform 2 is erythrocyte specific. Isoform 3 and isoform 4 are testis-specific.

**通路** Carbohydrate metabolism; hexose metabolism.

疾病相关 Hexokinase deficiency

Neuropathy, hereditary motor and sensory, Russe type

序列相似性 Belongs to the hexokinase family.

Contains 2 hexokinase domains.

结**构域** The N- and C-terminal halves of this hexokinase show extensive sequence similarity to each

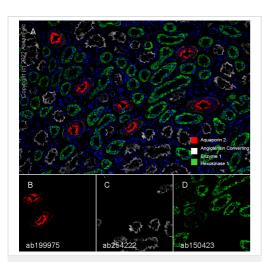
other. The catalytic activity is associated with the C-terminus while regulatory function is

associated with the N-terminus. Each domain can bind a single glucose and Gluc-6-P molecule.

细胞定位 Mitochondrion outer membrane. Its hydrophobic N-terminal sequence may be involved in

membrane binding.

### 图片



Multiplex immunohistochemistry - Anti-Hexokinase 1 antibody [EPR10134(B)] - Mitochondrial Outer Membrane Marker (ab150423)

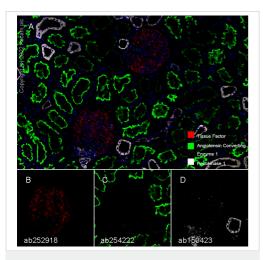
Fluorescence multiplex immunohistochemical analysis of the human kidney (Formalin/PFA-fixed paraffin-embedded sections).

Panel A: merged staining of anti-Hexokinase 1 (ab150423, green; Opal™690), anti-Angiotensin Converting Enzyme 1 (ab254222, gray; Opal™520) and anti-Aquaporin 2 (ab199975, red; Opal™570) on human kidney. Panel B: anti-Aquaporin 2 stained on collecting tubules. Panel C: anti-Angiotensin Converting Enzyme 1 stained on proximal tubules. Panel D: anti-Hexokinase 1 stained on distal tubules and collecting tubules. Opal Polymer HRP Ms + Rb was used as a secondary antibody.

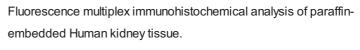
The section was incubated in three rounds of staining: in the order of ab150423 at 1/250 dilution (4.224  $\mu$ g/ml), **ab254222** at 1/4000 dilution (0.141  $\mu$ g/ml) and **ab199975** at 1/4000 dilution (0.152  $\mu$ g/ml) for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system.

The immunostaining was performed on a Leica Biosystems BOND<sup>®</sup> RX instrument with an Opal<sup>™</sup> 4-color kit. Image acquisition was performed with Leica SP8 confocal microscope.

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins. DAPI (blue) was used as a nuclear counter stain.



Multiplex immunohistochemistry - Anti-Hexokinase 1 antibody [EPR10134(B)] - Mitochondrial Outer Membrane Marker (ab150423)



Panel A: Merged staining of anti-Hexokinase 1 (gray; Opal™690), anti-Angiotensin Converting Enzyme 1 (green; Opal™520) and anti-Tissue Factor (red; Opal™570) on human kidney.

Panel B: Anti-Tissue Factor stained on renal glomeruli.

Panel C: Anti-Angiotensin Converting Enzyme 1 stained on proximal tubules.

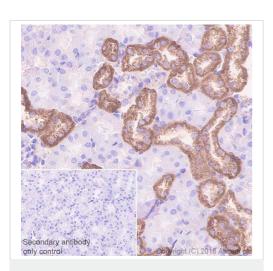
Panel D: Anti-Hexokinase 1 stained on distal tubules.

The section was incubated in three rounds of staining: in the order of ab150423, <u>ab254222</u>, and <u>ab252918</u> for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal<sup>™</sup> 4-color kit. Image acquisition was performed with Leica SP8 confocal microscope. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins. Counterstained with DAPI.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Hexokinase 1 antibody
[EPR10134(B)] - Mitochondrial Outer Membrane
Marker (ab150423)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Rat kidney tissue sections labeling Hexokinase 1 with purified ab150423 at 1/50 dilution (4.14 µg/mL). Perform heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0). ImmunoHistoProbe one step HRP Polymer (ready to use) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.

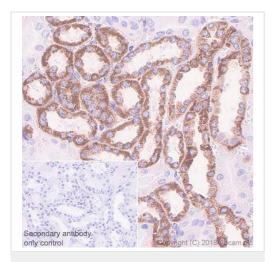


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Hexokinase 1 antibody

[EPR10134(B)] - Mitochondrial Outer Membrane

Marker (ab150423)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Mouse kidney tissue sections labeling Hexokinase 1 with purified ab150423 at 1/50 dilution (4.14  $\mu$ g/mL). Perform heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0). ImmunoHistoProbe one step HRP Polymer (ready to use) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Hexokinase 1 antibody

[EPR10134(B)] - Mitochondrial Outer Membrane

Marker (ab150423)

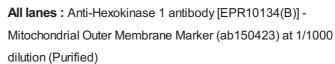
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human kidney tissue sections labeling Hexokinase 1 with purified ab150423 at 1/50 dilution (4.14 µg/mL). Perform heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0). ImmunoHistoProbe one step HRP Polymer (ready to use) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



Western blot - Anti-Hexokinase 1 antibody

[EPR10134(B)] - Mitochondrial Outer Membrane

Marker (ab150423)



Lane 1: Human brain lysate

Lane 2: Mouse brain lysate

Lane 3: Rat brain lysate

Lane 4: HEK-293 (Human embryonic kidney epithelial cell) whole

cell lysate

Lane 5 : MCF7 (Human breast adenocarcinoma epithelial cell)

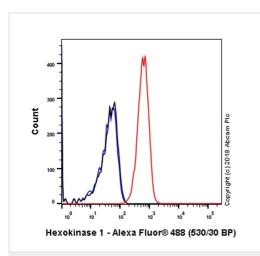
whole cell lysate

#### Secondary

All lanes: Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000

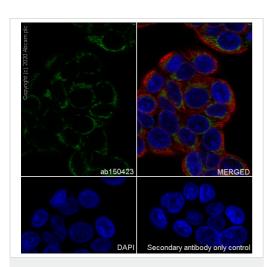
dilution

Predicted band size: 102 kDa



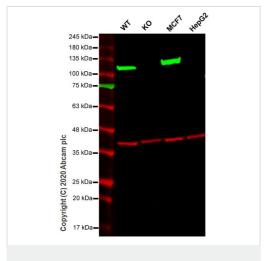
Flow Cytometry (Intracellular) - Anti-Hexokinase 1 antibody [EPR10134(B)] - Mitochondrial Outer Membrane Marker (ab150423)

Intracellular Flow Cytometry analysis of K-562 (Human chronic myelogenous leukemia lymphoblast) cells labeling Hexokinase 1 with Purified ab150423 at 1/20 dilution (10 µg/ml) (Red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit IgG (Alexa Fluor® 488 ,ab150077) secondary antibody was used at 1/2000. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).



Immunocytochemistry/ Immunofluorescence - Anti-Hexokinase 1 antibody [EPR10134(B)] -Mitochondrial Outer Membrane Marker (ab150423)

Immunocytochemistry analysis of MCF7 (Human breast adenocarcinoma epithelial cell) cells labeling Hexokinase 1 with Purified ab150423 at 1:50 dilution (4.1  $\mu$ g/ml). Cells were fixed in 100% Methanol and permeabilized with 0.1% tritonX-100. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1:200 (2.5  $\mu$ g/ml). Goat anti rabbit lgG (Alexa Fluor® 488,ab150077) was used as the secondary antibody at 1:1000 (2  $\mu$ g/ml) dilution. DAPI (blue) was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



Western blot - Anti-Hexokinase 1 antibody

[EPR10134(B)] - Mitochondrial Outer Membrane

Marker (ab150423)

**All lanes :** Anti-Hexokinase 1 antibody [EPR10134(B)] - Mitochondrial Outer Membrane Marker (ab150423) at 1/1000 dilution

Lane 1: Wild-type HEK-293T cell lysate
Lane 2: HK1 knockout HeLa cell lysate

Lane 3 : MCF7 cell lysate

Lane 4 : HEPG2 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 102 kDa Observed band size: 102 kDa

**Lanes 1-4:** Merged signal (red and green). Green - ab150423 observed at 102 kDa. Red - loading control, <u>ab8245</u> observed at 37 kDa.

ab150423 Anti-Hexokinase 1 antibody [EPR10134(B)] - Mitochondrial was shown to specifically react with Hexokinase 1 in wild-type HEK293T cells. Loss of signal was observed when knockout cell line <a href="mailto:ab267279">ab267279</a> (knockout cell lysate <a href="mailto:ab257161">ab257161</a>) was used. Wild-type and Hexokinase 1 knockout samples were subjected to SDS-PAGE. ab150423 and Anti-GAPDH antibody [6C5] - Loading Control (<a href="mailto:ab8245">ab8245</a>) 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 (<a href="mailto:ab216773">ab216773</a>) and Goat anti-Mouse lgG H&L (IRDye® 680RD) preadsorbed (<a href="mailto:ab216776">ab216773</a>) secondary antibodies at 1 in 10000 dilution for 1 hour at room temperature before imaging.



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