abcam

ab228819

Anti-Hexokinase II antibody [EPR20839] - BSA and Azide free

Anti-Hexokinase II antibody [EPR20839] - BSA and Azide free (ab228819) is a rabbit recombinant monoclonal antibody provided in a PBS only buffer for easy conjugation. Suitable for **Western Blot, Flow Cytometry (Intra), IP, IHC-P, ICC/IF** in **Human, Mouse, Rat**.

- KO validated for confirmed specificity
- BSA, sodium azide, and glycerol-free for easy conjugation
- Biophysical QC for unrivalled batch-batch consistency

Recombinant | RabMAb | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	EPR20839
Purification technique	Affinity purification Protein A
Concentration	1.044 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

ICC/IF

Tested

Species	Mouse, Human
Dilution info	-
Notes	-

Expected

Species	Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Not recommended

Species	Recombinant full length protein - Human
Dilution info	-
Notes	-

IHC-P

Tested

Species	Human
Dilution info	-
Notes	Not recommended for rat and mouse tissues. Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Not recommended

Species	Mouse, Rat, Recombinant full length protein - Human
Dilution info	-
Notes	-

IP

Tested

Species	Human
Dilution info	-
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Not recommended

Species	Recombinant full length protein - Human
Dilution info	-
Notes	-

WB

Tested

Species	Mouse, Rat, Human, Recombinant full length protein - Human
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.

Notes	-

Not recommended

Species	Recombinant full length protein - Human
Dilution info	-
Notes	-

Target data

See full target information HK2

Function	Catalyzes the phosphorylation of hexose, such as D-glucose and D-fructose, to hexose 6-phosphate (D-glucose 6-phosphate and D-fructose 6-phosphate, respectively) (PubMed:23185017, PubMed:26985301, PubMed:29298880). Mediates the initial step of glycolysis by catalyzing phosphorylation of D-glucose to D-glucose 6-phosphate (PubMed:29298880). Plays a key role in maintaining the integrity of the outer mitochondrial membrane by preventing the release of apoptogenic molecules from the intermembrane space and subsequent apoptosis (PubMed:18350175).

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

What is this antibody validated in?
Anti-Hexokinase II antibody [EPR20839] - BSA and Azide free (ab228819) is a rabbit recombinant monoclonal antibody and is validated for use in Western Blot (WB), Flow Cytometry (Intra), Flow Cytometry (Flow Cyt), Immunoprecipitation (IP), Immunohistochemistry (IHC-P), Immunocytochemistry/immunofluorescence (ICC/IF) in Human, Mouse, Rat samples.

What is the molecular weight of Hexokinase II?
Anti-Hexokinase II [EPR20839] - BSA and Azide free (ab228819) specifically detects a band for Hexokinase II (UniProt: P52789) at a molecular weight of 102kDa.

Specificity confirmed
The specificity of Anti-Hexokinase II antibody [EPR20839] - BSA and Azide free (ab228819) has been confirmed by Western blot testing in HK2 Knockout HEK-293 cell line.

Other related products
We have a range of other formats of antibody clone [EPR20839] also available for your convenience: ab209847, Carrier free - ab228819, Alexa Fluor® 647 - ab237314, HRP - ab237315, ab323242, Carrier free - ab323251

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

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Conjugation ready
Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with 1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

Compatibility
This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

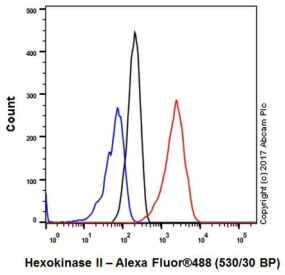
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

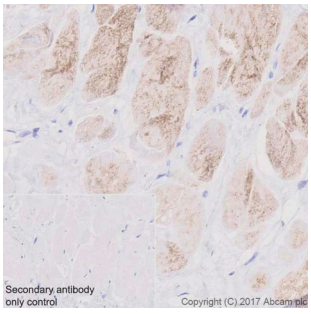
8 product images



Flow Cytometry (Intracellular) - Anti-Hexokinase II antibody [EPR20839] - BSA and Azide free (ab228819)

Hexokinase II Flow Cytometry (Intracellular) staining using rabbit Anti-Hexokinase II antibody

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol-permeabilized HeLa (human epithelial cell line from cervix adenocarcinoma) cell line labeling Hexokinase II with ab209847 at 1/60 dilution (red) compared with a Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730) (black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) at 1/2000 dilution was used as the secondary antibody.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab209847).



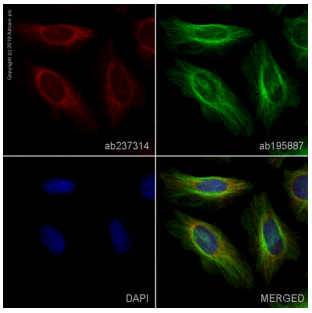
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Hexokinase II antibody [EPR20839] - BSA and Azide free (ab228819)

Hexokinase II Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Hexokinase II antibody

Immunohistochemical analysis of paraffin-embedded human heart tissue labeling Hexokinase II with ab209847 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Granularly cytoplasmic staining in human heart (PMID: 4058069; PMID: 26722360) is observed. Counter stained with Hematoxylin.
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab209847).

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

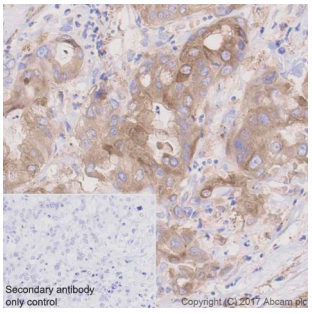


Immunocytochemistry/ Immunofluorescence - Anti-Hexokinase II antibody [EPR20839] - BSA and Azide free (ab228819)

Hexokinase II Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Hexokinase II antibody

Clone EPR20839 (ab228819) has been successfully conjugated by Abcam. This image was generated using Anti-Hexokinase II antibody [EPR20839] (Alexa Fluor® 647). Please refer to ab237314 for protocol details.
ab237314 staining Hexokinase II in HeLa cells. The cells were fixed with 4% formaldehyde (10 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with ab237314 at 1/1000 dilution (shown in red) and ab195887, Mouse monoclonal to alpha Tubulin (Alexa Fluor® 488), at 1/250 dilution (shown in green). Nuclear DNA was labelled with DAPI (shown in blue).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



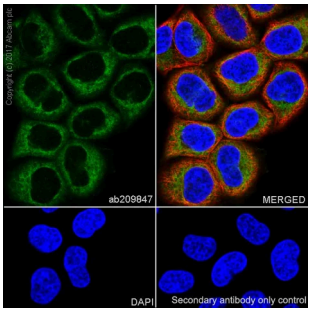
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Hexokinase II antibody [EPR20839] - BSA and Azide free (ab228819)

Hexokinase II Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Hexokinase II antibody

Immunohistochemical analysis of paraffin-embedded human pancreatic adenocarcinoma tissue labeling Hexokinase II with ab209847 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Strong cytoplasmic staining in human pancreatic adenocarcinoma (PMID: 26137268) is observed. Counter stained with Hematoxylin.
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab209847).

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



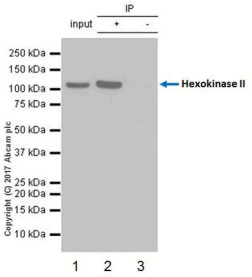
Immunocytochemistry/ Immunofluorescence - Anti-Hexokinase II antibody [EPR20839] - BSA and Azide free (ab228819)

Hexokinase II Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Hexokinase II antibody

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (human cervix adenocarcinoma epithelial cell) cells labeling Hexokinase II with ab209847 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). Cytoplasmic staining in HeLa cells.
The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (ab195889) (red) at 1/200 dilution.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab209847).



Immunoprecipitation - Anti-Hexokinase II antibody [EPR20839] - BSA and Azide free (ab228819)

Hexokinase II was immunoprecipitated from 0.35 mg HeLa (humanepithelial cell line from cervix adenocarcinoma) whole cell lysate with ab209847 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab209847 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/10000 dilution.
Lane 1: HeLa whole cell lysate 10 µg (Input).
Lane 2: ab209847 IP in HeLa whole cell lysate.
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab209847 in HeLa whole cell lysate.

Exposure time: 30 seconds.

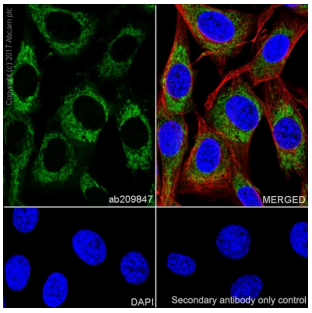
Blocking and dilution buffer and concentration: 5% NFDM/TBST.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab209847).

All lanes:
Immunoprecipitation - Anti-Hexokinase II antibody [EPR20839] (ab209847)

Predicted band size: 102 kDa

Observed band size: 102 kDa



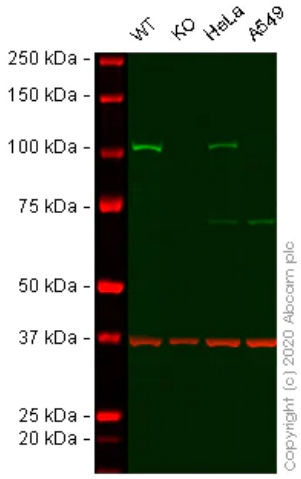
Immunocytochemistry/ Immunofluorescence - Anti-Hexokinase II antibody [EPR20839] - BSA and Azide free (ab228819)

Hexokinase II Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Hexokinase II antibody

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized NIH/3T3 (mouse embryonic fibroblast cell line) cells labeling Hexokinase II with ab209847 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). Cytoplasmic staining in NIH/3T3 cells.
The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (ab195889) (red) at 1/200 dilution.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab209847).



Western blot - Anti-Hexokinase II antibody [EPR20839] - BSA and Azide free (ab228819)

Hexokinase II Western blot staining using rabbit Anti-Hexokinase II antibody

This data was developed using the same antibody clone in a different buffer formulation (ab209847).
Lanes 1 - 4: Merged signal (red and green). Green - ab209847 observed at 110 kDa. Red - loading control ab8245 (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

ab209847 was shown to react with Hexokinase II in wild-type HEK-293 cells in western blot with loss of signal observed in HK2 knockout sample. Wild-type and HK2 knockout HEK-293 cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before incubation with ab209847 and ab8245 (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at a 1 in 1000 Dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

All lanes:
Western blot - Anti-Hexokinase II antibody [EPR20839] (ab209847) at 1/1000 dilution

Lane 1:
Wild-type HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate at 20 µg

Lane 2:
Western blot - Human HK2 (Hexokinase II) knockout HEK-293 cell lysate (ab281353) at 20 µg

Lane 3:
HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate at 20 µg

Lane 4:
A549 (Human lung carcinoma cell line) whole cell lysate at 20 µg

Performed under reducing conditions.

Predicted band size: 102 kDa

Observed band size: 110 kDa

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.