abcam

ab218121

Anti-PFKFB3 antibody [EPR12594] - BSA and Azide free

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	EPR12594
Purification technique	Affinity purification Protein A
Concentration	1.057 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Tested

Species	Human, Mouse, Rat
Dilution info	-

Notes	-

WB

Tested

Species	Human, Rat, Mouse
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	-
Notes	-

Not recommended

Species	Rat, Mouse
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-
Notes	ab199376 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Expected

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

Notes	-

IHC-P

Tested

Species	Human
Dilution info	-
Notes	-

Not recommended

Species	Rat, Mouse
Dilution info	-
Notes	-

Target data

See full target information PFKFB3

Function	Catalyzes both the synthesis and degradation of fructose 2,6-bisphosphate.

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

ab218121 is the carrier-free version of ab181861.

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.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	PFKFB3 also known as 6-phosphofructo-2-kinase/fructose-26-bisphosphatase 3 serves a significant role in regulating glycolysis. This bifunctional enzyme weighs approximately 58 kDa and manages levels of fructose-26-bisphosphate a potent activator of another enzyme called phosphofructokinase-1. PFKFB3 expression occurs in various tissues but is especially high in areas with active cell proliferation like the brain and tumors. This enzyme's activity affects glycolytic flux critical for energy production and biosynthesis especially under conditions requiring rapid energy supply.
Biological function summary	PFKFB3 regulates cellular energy metabolism serving as a switch for converting carbohydrates into energy and building blocks for growth. This enzyme is part of a glycolytic complex coordinating energy needs during proliferation and survival. Its activity is important in processes demanding increased energy and anabolic precursors such as cell division. The enzyme's modulation affects fructose-26-bisphosphate levels influencing cellular responses to stress and nutrient availability directly impacting metabolic adaptation.
Pathways	PFKFB3 integrates into the glycolytic pathway and the pentose phosphate pathway. PFKFB3's control over fructose-26-bisphosphate levels influences phosphofructokinase-1 modulating the glycolytic pathway's rate. This modulation impacts energy production efficiency and the provision of metabolites required for nucleotide synthesis linking to the pentose phosphate pathway. Proline-rich AKT substrate of 40 kDa (PRAS40) and AKT may also interact indirectly to regulate PFKFB3 activity in these metabolic pathways.

Associated diseases and disorders

PFKFB3 links strongly to cancer and diabetes. Its upregulation in cancerous tissues supports tumor growth by promoting glycolysis known as the Warburg effect. Cancer-associated proteins such as KRAS often rely on PFKFB3's glycolytic control to sustain proliferative signaling. Additionally in diabetes altered PFKFB3 activity can contribute to insulin resistance due to disrupted metabolic homeostasis with insulin pathway proteins like IRS1 potentially interacting with it in this context.

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

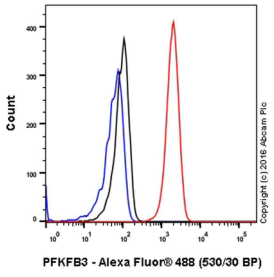
We do not recommend this combination. It is not covered by our product promise.

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.

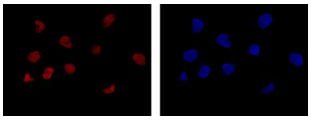
10 product images



Flow Cytometry (Intracellular) - Anti-PFKFB3 antibody [EPR12594] - BSA and Azide free (ab218121)

PFKFB3 Flow Cytometry (Intracellular) staining using rabbit Anti-PFKFB3 antibody

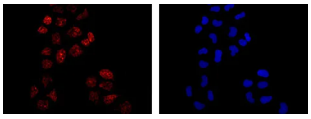
Intracellular Flow Cytometry analysis of A431 (human epidermoid carcinoma) cells labeling PFKFB3 with purified ab181861 at 1/210 dilution (10ug/ml) (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. A Goat anti rabbit IgG (Alexa Fluor® 488) (1/2000 dilution) was used as the secondary antibody. Rabbit monoclonal IgG (Black) was used as the isotype control, cells without incubation with primary antibody and secondary antibody (Blue) was used as the unlabeled control.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab181861).



Immunocytochemistry/ Immunofluorescence - Anti-PFKFB3 antibody [EPR12594] - BSA and Azide free (ab218121)

PFKFB3 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-PFKFB3 antibody

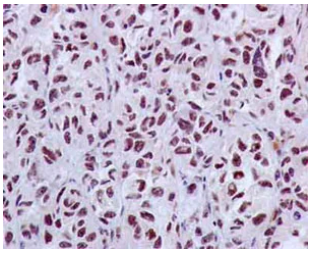
Immunofluorescent analysis of 4% paraformaldehyde-fixed A431 cells labeling PFKFB3 with ab181861 at 1/100 dilution, followed by Goat anti rabbit IgG (Alexa Fluor®555) at 1/200 dilution. Counter stained with Dapi (blue).
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab181861).



Immunocytochemistry/ Immunofluorescence - Anti-PFKFB3 antibody [EPR12594] - BSA and Azide free (ab218121)

PFKFB3 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-PFKFB3 antibody

Immunofluorescent analysis of acetone-fixed HeLa cells labeling PFKFB3 with ab181861 at 1/100 dilution, followed by Goat anti rabbit IgG (Alexa Fluor®555) at 1/200 dilution. Counter stained with Dapi (blue).
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab181861).

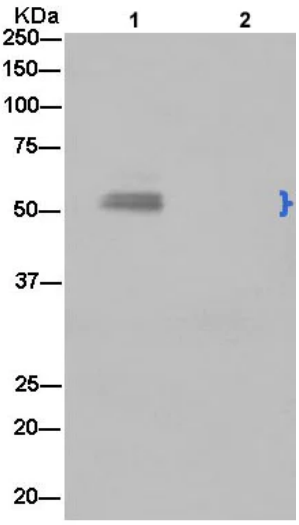


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PFKFB3 antibody [EPR12594] - BSA and Azide free (ab218121)

PFKFB3 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-PFKFB3 antibody

This IHC data was generated using the same anti-PFKFB3 antibody clone, EPR12594, in a different buffer formulation (cat# ab181861).
Immunohistochemical analysis of paraffin-embedded human melanoma tissue labeling PFKFB3 with ab181861 at 1/50 dilution followed by prediluted HRP Polymer for Rabbit IgG. Counter stained with Hematoxylin.

Perform heat mediated antigen retrieval with EDTA buffer pH 9 before commencing with IHC staining protocol.



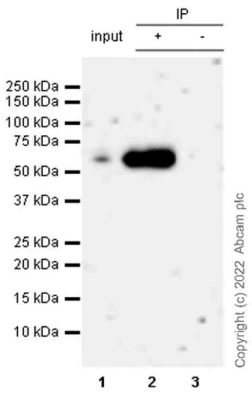
Immunoprecipitation - Anti-PFKFB3 antibody [EPR12594] - BSA and Azide free (ab218121)

Western blot analysis of PFKFB3 in HeLa cell lysate immunoprecipitated using ab181861 at 1/50 dilution (Lane 1). Lane 2: Negative control.
Secondary antibody: Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/1500 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab181861).

All lanes:
Immunoprecipitation - Anti-PFKFB3 antibody [EPR12594] (ab181861)

Predicted band size: 60 kDa



Immunoprecipitation - Anti-PFKFB3 antibody [EPR12594] - BSA and Azide free (ab218121)

PFKFB3 was immunoprecipitated from 0.35 mg of AR42J (rat pancreatic tumor epithelial cell) whole cell lysate with ab181861 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab181861 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/5000 dilution. Lane 1: AR42J (rat pancreatic tumor epithelial cell) whole cell lysate 10 µg (Input). Lane 2: ab181861 IP in AR42J whole cell lysate Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab181861 in AR42J whole cell lysate Blocking and dilution buffer and concentration: 5% NFDM/TBST. Exposure time: 180 seconds. This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab181861).
All lanes:
Immunoprecipitation - Anti-PFKFB3 antibody [EPR12594] (ab181861)

Lane 1:
AR42J (rat pancreatic tumor epithelial cell) whole cell lysate at 10 µg

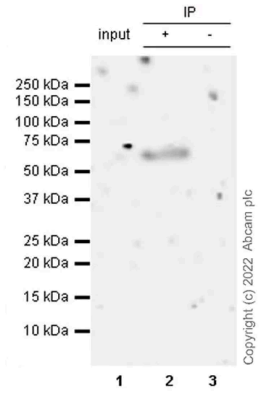
Lane 2:
Anti-PFKFB3 antibody IP in AR42J whole cell lysate

Secondary

All lanes:
Immunoprecipitation - VeriBlot for IP Detection Reagent (HRP) (ab131366) at 1/5000 dilution

Predicted band size: 60 kDa

Observed band size: 60 kDa



Immunoprecipitation - Anti-PFKFB3 antibody [EPR12594] - BSA and Azide free (ab218121)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab181861).
PFKFB3 was immunoprecipitated from 0.35 mg of Mouse skin tissue lysate with ab181861 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab181861 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/5000 dilution.

Lane 1: Mouse skin tissue lysate 10 µg (Input)

Lane 2: ab181861 at 1/30 IP in Mouse skin tissue lysate

Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab181861 in Mouse skin tissue lysate

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

This blot was developed using a high sensitivity ECL substrate. The high-sensitivity ECL substrate used allows for the detection of proteins in the mid-femtogram range.

All lanes:
Immunoprecipitation - Anti-PFKFB3 antibody [EPR12594] (ab181861) at 1/1000 dilution

Lane 1:
Mouse skin tissue lysate at 10 µg

Lane 2:
ab181861 at 1/30 IP in Mouse skin tissue lysate at 1/30 dilution

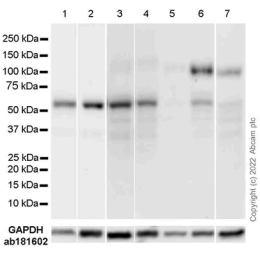
Secondary

All lanes:
Immunoprecipitation - VeriBlot for IP Detection Reagent (HRP) (ab131366) at 1/5000 dilution

Developed using the ECL technique.

Observed band size: 60 kDa

Exposure time: 180s



Western blot - Anti-PFKFB3 antibody [EPR12594] - BSA and Azide free (ab218121)

PFKFB3 Western blot staining using rabbit Anti-PFKFB3 antibody

Exposure time: Lane 1-2: 26 seconds, Lane 3-7: 48 seconds
Blocking buffer and concentration: 5% NFDM/TBST

Lane 3-7 were developed using a high sensitivity ECL substrate.

The expression level of PFKFB3 is upregulated during 3T3-L1 differentiation (PMID: 16306349).
The band at approximately 110 kDa is likely to be PFKFB3 dimer (PMID: 31889092).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab181861).

All lanes:
Western blot - Anti-PFKFB3 antibody [EPR12594] (ab181861) at 1/1000 dilution

Lane 1:

A431 (human epidermoid carcinoma epithelial cell), whole cell lysate at 20 µg

Lane 2:
bEnd.3 (mouse brain endothelial cell), whole cell lysate at 20 µg

Lane 3:
NIH/3T3 (mouse embryonic fibroblast), whole cell lysate at 20 µg

Lane 4:
4T1 (mouse mammary gland carcinoma epithelial cell), whole cell lysate at 20 µg

Lane 5:
Undifferentiated 3T3-L1 (mouse embryonic fibroblast), whole cell lysate at 20 µg

Lane 6:
3T3-L1 (mouse embryonic fibroblast) differentiated into adipocyte-like cells, whole cell lysate at 20 µg

Lane 7:
C2C12 (mouse myoblast), whole cell lysate at 20 µg

Secondary

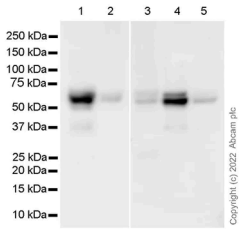
All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 60 kDa

Observed band size: 60 kDa



Western blot - Anti-PFKFB3 antibody [EPR12594] - BSA and Azide free (ab218121)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab181861).
Blocking and diluting buffer and concentration: 5% NFDM/TBST.

This blot was developed using a high sensitivity ECL substrate. The high-sensitivity ECL substrate used allows for the detection of proteins in the mid-femtogram range.

All lanes:
Western blot - Anti-PFKFB3 antibody [EPR12594] (ab181861) at 1/1000 dilution

Lane 1:
A431 (human epidermoid carcinoma epithelial cell) whole cell lysate at 20 µg

Lane 2:
Mouse skin tissue lysate at 20 µg

Lane 3:
Rat breast tissue lysate at 20 µg

Lane 4:
AR42J (rat pancreatic tumor epithelial cell) whole cell lysate at 20 µg

Lane 5:
L6 (rat skeletal muscle myoblast) whole cell lysate at 20 µg

Secondary

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

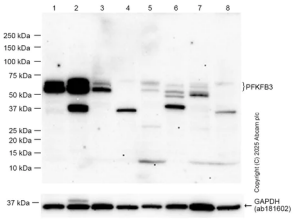
Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 60 kDa

Observed band size: 60 kDa

Exposure time: 15s



Western blot - Anti-PFKFB3 antibody [EPR12594] - BSA and Azide free (ab218121)

PFKFB3 Western blot staining using rabbit Anti-PFKFB3 antibody

This data was developed using ab181861, the same antibody clone in a different buffer formulation.
Low expression: kidney tissue (PMID: 34660743).

In Western blot, Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602) staining at 1/1000000 dilution.

All lanes:
Western blot - Anti-PFKFB3 antibody [EPR12594] (ab181861) at 1/1000 dilution

Lane 1:
Mouse lung tissue lysate at 20 µg

Lane 2:
Mouse liver tissue lysate at 20 µg

Lane 3:
Mouse heart tissue lysate at 20 µg

Lane 4:
Mouse kidney tissue lysate at 20 µg

Lane 5:
Rat lung tissue lysate at 20 µg

Lane 6:
Rat liver tissue lysate at 20 µg

Lane 7:
Rat heart tissue lysate at 20 µg

Lane 8:
Rat kidney tissue lysate at 20 µg

Secondary

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

Developed using the ECL technique.

Observed band size: 60 kDa

Exposure time: 180s

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