abcam

# ab181861

# Anti-PFKFB3 antibody [EPR12594]

Anti-PFKFB3 antibody [EPR12594] (ab181861) is a rabbit monoclonal antibody detecting PFKFB3 in **Western Blot, Flow Cytometry (Intra), IP, IHC-P, ICC/IF**. Suitable for **Human, Mouse, Rat**.

- KO validated for confirmed specificity
- Biophysical QC for unrivalled batch-batch consistency
- Over 70 publications

Recombinant | RabMAb | KO Validated | 20ul selling size

## Key facts

| | |
|---|---|
| **Isotype** | IgG |
| **Host species** | Rabbit |
| **Storage buffer** | Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA |
| **Form** | Liquid |
| **Clonality** | Monoclonal |
| **Immunogen** | The exact immunogen used to generate this antibody is proprietary information. |
| **Clone number** | EPR12594 |
| **Purification technique** | Affinity purification Protein A |
| **Concentration** | 0.544 mg/mL The concentration of this product may be batch-dependent<br>Batch concentration finder → |

## Reactivity data

### IP

**Tested**

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | 1/50 |
| **Notes** | - |

| | |
|---|---|
| **Species** | Mouse |
| **Dilution info** | 1/50 |
| **Notes** | - |

| | |
|---|---|
| **Species** | Rat |
| **Dilution info** | 1/50 |
| **Notes** | - |

## WB

### Tested

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | 1/1000 - 1/10000 |
| **Notes** | - |

| | |
|---|---|
| **Species** | Rat |
| **Dilution info** | 1/1000 - 1/10000 |
| **Notes** | - |

| | |
|---|---|
| **Species** | Mouse |
| **Dilution info** | 1/1000 - 1/10000 |
| **Notes** | - |

## ICC/IF

### Tested

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | 1/100 |
| **Notes** | - |

### Not recommended

| | |
|---|---|
| **Species** | Mouse, Rat |
| **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** | - |

## IHC-P

### Tested

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | 1/50 |
| **Notes** | Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. |

### Not recommended

| | |
|---|---|
| **Species** | Mouse, Rat |
| **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** | Conditional Ambient |
| **Appropriate short-term storage duration** | 1-2 weeks |
| **Appropriate short-term storage conditions** | +4°C |
| **Appropriate long-term storage conditions** | -20°C |
| **Aliquoting information** | Upon delivery aliquot |
| **Storage information** | Avoid freeze / thaw cycle |

## Notes

**What is this antibody validated in?**
Anti-PFKFB3 antibody [EPR12594] (ab181861) 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 PFKFB3?**
Anti-PFKFB3 [EPR12594] (ab181861) specifically detects a band for PFKFB3 (UniProt: Q16875) at a molecular weight of 60kDa.

**Trusted by the scientific community**
Anti-PFKFB3 [EPR12594] (ab181861) was first used in a scientific publication in 2014 and has been cited over 70 times in peer-reviewed journals.

**Reviewed by scientists**
Anti-PFKFB3 [EPR12594] (ab181861) has over 5 independent reviews from customers.

**Specificity confirmed**
The specificity of Anti-PFKFB3 antibody [EPR12594] (ab181861) has been confirmed by Western blot testing in PFKFB3 Knockout HAP1 cells.

**Other related products**
We have a range of other formats of antibody clone [EPR12594] also available for your convenience: ab181861, Alexa Fluor® 647 - ab203651, Alexa Fluor® 488 - ab203984, Carrier free - ab218121

**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.

**Trial sizes available!**
Test your antibody or perform pre-screening before committing to a larger quantity. Sold in 20µl. Discover our selection of trial-size antibodies.

## 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.

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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.

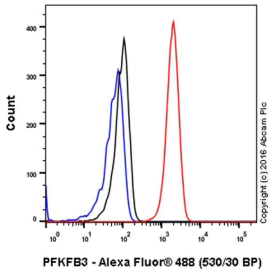
In the unlikely event of one of our products not working as expected, you are covered by our product promise.

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

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## 10 product images

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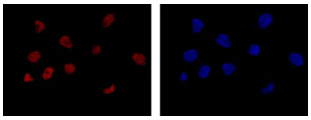



### Flow Cytometry (Intracellular) - Anti-PFKFB3 antibody [EPR12594] (ab181861)

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.

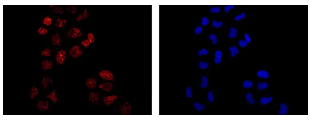
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## Immunocytochemistry/ Immunofluorescence - Anti-PFKFB3 antibody [EPR12594] (ab181861)

PFKFB3 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-PFKFB3 antibody

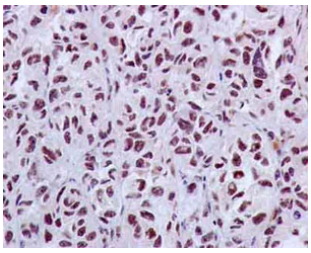
Immunofluorescent analysis of 4% paraformaldehyde-fixed A431 cells labeling PFKFB3 with ab181861 at 1/100 dilution, followed by Goat anti rabbit IgG (Alexa Fluor®555) at 1/200 dilution. Counter stained with Dapi (blue).



## Immunocytochemistry/ Immunofluorescence - Anti-PFKFB3 antibody [EPR12594] (ab181861)

PFKFB3 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-PFKFB3 antibody

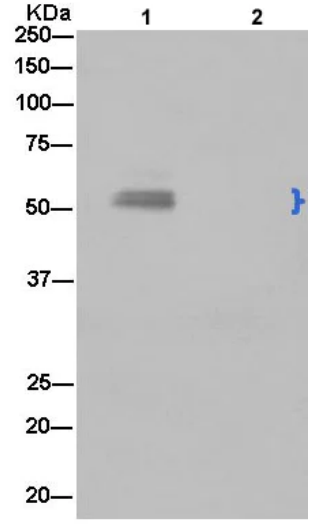
Immunofluorescent analysis of acetone-fixed HeLa cells labeling PFKFB3 with ab181861 at 1/100 dilution, followed by Goat anti rabbit IgG (Alexa Fluor®555) at 1/200 dilution. Counter stained with Dapi (blue).



## Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PFKFB3 antibody [EPR12594] (ab181861)

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

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.

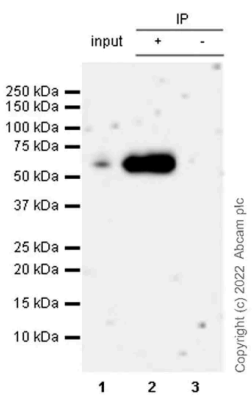



## Immunoprecipitation - Anti-PFKFB3 antibody [EPR12594] (ab181861)

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.

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

Predicted band size: 60 kDa




## Immunoprecipitation - Anti-PFKFB3 antibody [EPR12594] (ab181861)

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

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

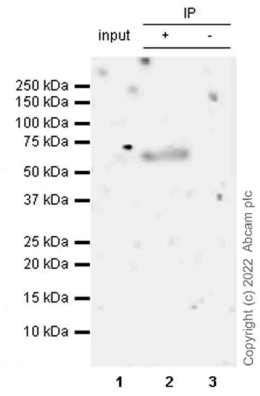
Lane 2:
Anti-PFKFB3 antibody IP in AR42J whole cell lysate

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] (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

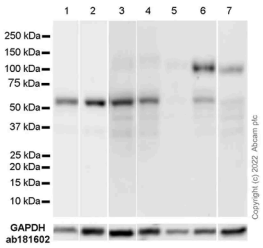
Secondary

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] (ab181861)

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).

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

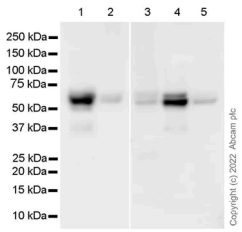
All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

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] (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

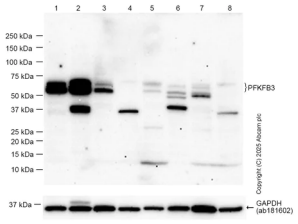
Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 60 kDa

Observed band size: 60 kDa

Exposure time: 15s




## Western blot - Anti-PFKFB3 antibody [EPR12594] (ab181861)

PFKFB3 Western blot staining using rabbit Anti-PFKFB3 antibody

**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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