

## Product datasheet

### Anti-PFKFB3 antibody [EPR12594] **ab181861**

KO VALIDATED

Recombinant

RabMAb

★★★★★ **6 Abreviews** **57 References** **12 Images**

#### Overview

<b>Product name</b>	Anti-PFKFB3 antibody [EPR12594]
<b>Description</b>	Rabbit monoclonal [EPR12594] to PFKFB3
<b>Host species</b>	Rabbit
<b>Tested applications</b>	<b>Suitable for:</b> Flow Cyt (Intra), WB, IP, ICC/IF, IHC-P
<b>Species reactivity</b>	<b>Reacts with:</b> Mouse, Rat, Human
<b>Immunogen</b>	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
<b>Positive control</b>	WB: Jurkat and HeLa whole cell lysate ( <b>ab150035</b> ); Human melanoma tissue; HeLa and A431 cells, Mouse skin tissue lysate, Rat breast tissue lysate, AR42 and L6 whole cell lysates, HAP1 whole cell lysate, AR42J (rat pancreatic tumor epithelial cell) whole cell lysate, IP: Mouse skin tissue lysate, AR42J, whole cell lysate ICC: HeLa, A431 cells IHC: human melanoma tissue Flow: A431 (human epidermoid carcinoma) cells,
<b>General notes</b>	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> <li>- High batch-to-batch consistency and reproducibility</li> <li>- Improved sensitivity and specificity</li> <li>- Long-term security of supply</li> <li>- Animal-free production</li> </ul> <p>For more information <a href="#">see here</a>.</p> <p>Our RabMAb<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <a href="#">RabMAb<sup>®</sup> patents</a>.</p>

#### Properties

<b>Form</b>	Liquid
<b>Storage instructions</b>	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.
<b>Storage buffer</b>	Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
<b>Purity</b>	Protein A purified
<b>Clonality</b>	Monoclonal
<b>Clone number</b>	EPR12594

Isotype

IgG

## Applications

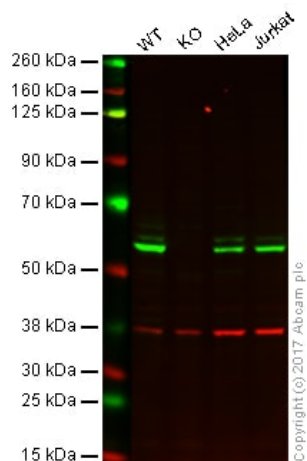
**The Abpromise guarantee** Our **Abpromise guarantee** covers the use of ab181861 in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		Use at an assay dependent concentration.
WB	★★★★★ (5)	1/1000 - 1/10000. Detects a band of approximately 58 kDa (predicted molecular weight: 60 kDa).
IP		1/50.
ICC/IF		1/100.
IHC-P		1/50. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

## Target

Function	Synthesis and degradation of fructose 2,6-bisphosphate.
Tissue specificity	Ubiquitous.
Sequence similarities	In the C-terminal section; belongs to the phosphoglycerate mutase family.
Post-translational modifications	Phosphorylation by AMPK stimulates activity.

## Images



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

**Lane 1:** Wild-type HAP1 whole cell lysate (20 µg)

**Lane 2:** PFKFB3 knockout HAP1 whole cell lysate (20 µg)

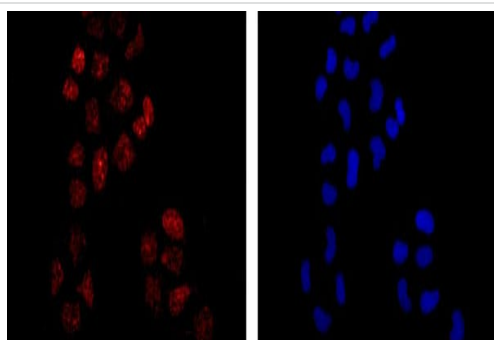
**Lane 3:** HeLa whole cell lysate (20 µg)

**Lane 4:** Jurkat whole cell lysate (20 µg)

**Lanes 1 - 4:** Merged signal (red and green). Green - ab181861 observed at 60 kDa. Red - loading control, **ab9484**, observed at 37 kDa.

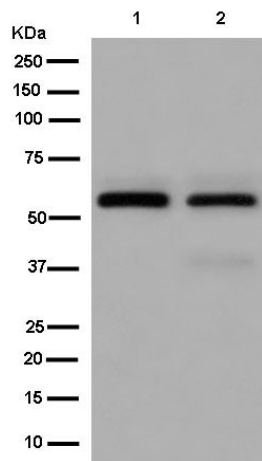
ab181861 was shown to specifically react with PFKFB3 in wild-type HAP1 cells as signal was lost in PFKFB3 knockout cells. Wild-type and PFKFB3 knockout samples were subjected to SDS-PAGE.

Ab181861 and **ab9484** (Mouse anti GAPDH loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/20000 dilution respectively. Blots were developed 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 1/20000 dilution for 1 hour at room temperature before imaging.



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

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).



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

**All lanes :** Anti-PFKFB3 antibody [EPR12594] (ab181861) at  
1/20000 dilution

**Lane 1 :** Jurkat cell lysate

**Lane 2 :** HeLa cell lysate

Lysates/proteins at 20 µg per lane.

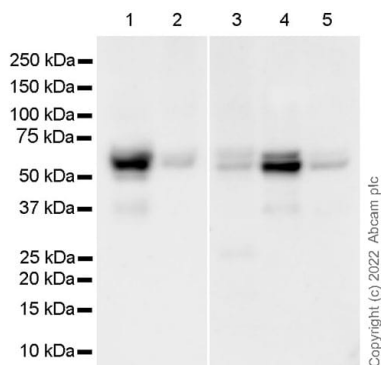
#### Secondary

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

**Predicted band size:** 60 kDa

**Observed band size:** 58 kDa

Blocking buffer: 5% NFDM/TBST



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

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

**Lane 1 :** A431 (human epidermoid carcinoma epithelial cell) whole  
cell lysate

**Lane 2 :** Mouse skin tissue lysate

**Lane 3 :** Rat breast tissue lysate

**Lane 4 :** AR42J (rat pancreatic tumor epithelial cell) whole cell  
lysate

**Lane 5 :** L6 (rat skeletal muscle myoblast) whole cell lysate

Lysates/proteins at 20 µg per lane.

#### Secondary

**All lanes :** 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

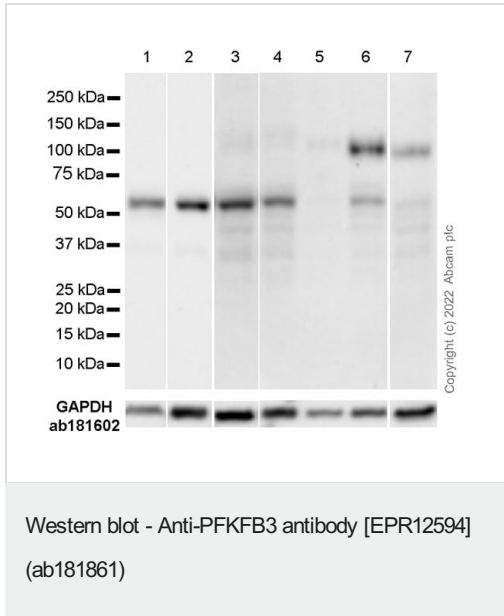
**Observed band size:** 60 kDa

**Exposure time:** 15 seconds

Blocking buffer: 5% NFDM/TBST

Exposure time: 15 seconds

This blot was developed using a high sensitivity ECL substrate.



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

**Lane 1 :** A431 (human epidermoid carcinoma epithelial cell), whole cell lysate

**Lane 2 :** bEnd.3 (mouse brain endothelial cell), whole cell lysate

**Lane 3 :** NIH/3T3 (mouse embryonic fibroblast), whole cell lysate

**Lane 4 :** 4T1 (mouse mammary gland carcinoma epithelial cell), whole cell lysate

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

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

**Lane 7 :** C2C12 (mouse myoblast), whole cell lysate

Lysates/proteins at 20 µg per lane.

### Secondary

**All lanes :** 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:** Lane 1-2: 26 seconds, Lane 3-7: 48 seconds

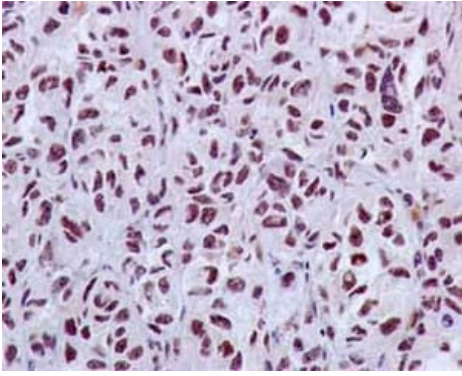
**Blocking buffer and concentration:** 5% NFDM/TBST

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

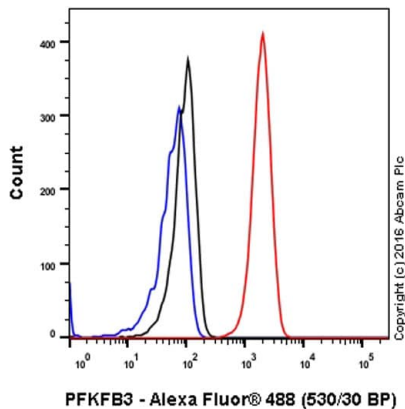
(PMID: 31889092).



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

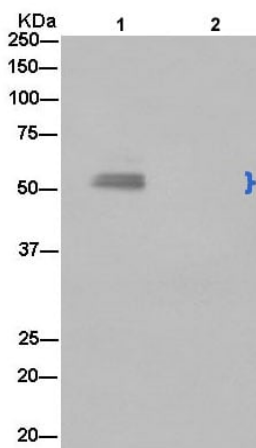
Immunohistochemical analysis of paraffin-embedded human melanoma tissue labeling PFKFB3 with ab181861 at 1/50 dilution followed by prediluted HRP Polymer for Rabbit IgG. Counter stained with Hematoxylin.

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



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

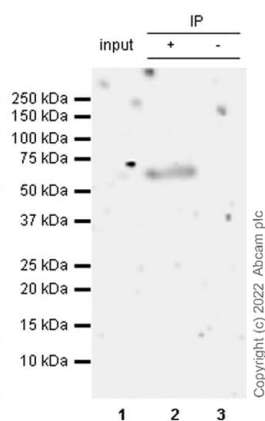
Intracellular Flow Cytometry analysis of A431 (human epidermoid carcinoma) cells labeling PFKFB3 with purified ab181861 at 1/210 dilution (10ug/ml) (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. A Goat anti rabbit IgG (Alexa Fluor® 488) (1/2000 dilution) was used 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.



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.



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).

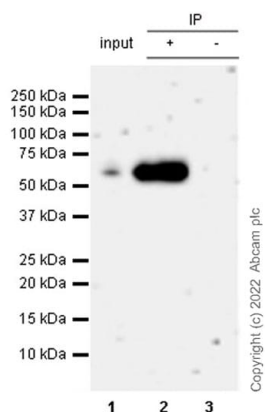
**Lane 2:** ab181861 IP in Mouse skin tissue lysate.

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

**Blocking and dilution buffer and concentration:** 5%  
NFDm/TBST.

**Exposure time:** 180 seconds.

This blot was developed using a high sensitivity ECL substrate.



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.

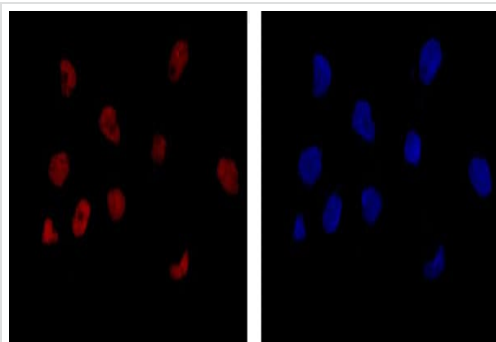
**Lane 1:** AR42J (rat pancreatic tumor epithelial cell) whole cell lysate 10 µg (Input).

**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.



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

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).

### Why choose a recombinant antibody?



**Research with confidence**  
Consistent and reproducible results



**Long-term and scalable supply**  
Recombinant technology



**Success from the first experiment**  
Confirmed specificity



**Ethical standards compliant**  
Animal-free production

Anti-PFKFB3 antibody [EPR12594] (ab181861)

**Please note:** All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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