abcam

# ab325016

# Anti-PFKP antibody [EPR28830-157]

Recombinant | RabMAb | KO Validated | 20ul selling size

## Key facts

| | |
|---|---|
| **Isotype** | IgG |
| **Host species** | Rabbit |
| **Storage buffer** | pH: 7.2 - 7.4 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** | EPR28830-157 |
| **Purification technique** | Affinity purification Protein A |
| **Concentration** | 0.521 mg/mL The concentration of this product may be batch-dependent<br>Batch concentration finder → |

## Reactivity data

## I-ELISA

### Tested

| | |
|---|---|
| **Species** | Synthetic peptide - Human |
| **Dilution info** | 62.5 ng/mL |
| **Notes** | - |

### Expected

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

## IP

### Tested

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | 1/30 |
| **Notes** | - |

### Expected

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

### Not recommended

| | |
|---|---|
| **Species** | Synthetic peptide - Human |
| **Dilution info** | - |
| **Notes** | - |

## Flow Cyt (Intra)

### Tested

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | 1/500 |
| **Notes** | - |

### Expected

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

| | |
|---|---|
| **Dilution info** | Use at an assay dependent concentration. |
| **Notes** | - |

### Not recommended

| | |
|---|---|
| **Species** | Synthetic peptide - Human |
| **Dilution info** | - |
| **Notes** | - |

## ICC/IF

### Tested

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | 1/200 |
| **Notes** | - |

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

### Expected

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

### Not recommended

| | |
|---|---|
| **Species** | Synthetic peptide - Human |
| **Dilution info** | - |
| **Notes** | - |

## WB

### Tested

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

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

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

### Not recommended

| | |
|---|---|
| **Species** | Synthetic peptide - Human |
| **Dilution info** | - |
| **Notes** | - |

## IHC-P

### Not recommended

| | |
|---|---|
| **Species** | Human, Mouse, Rat, Synthetic peptide - Human |
| **Dilution info** | - |
| **Notes** | - |

## Target data

See full target information PFKP

| | |
|---|---|
| **Function** | Catalyzes the phosphorylation of D-fructose 6-phosphate to fructose 1,6-bisphosphate by ATP, the first committing step of glycolysis. |

## 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

**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.

## 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**

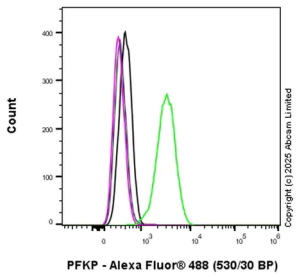
We do not recommend this combination. It is not covered by our product promise.

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

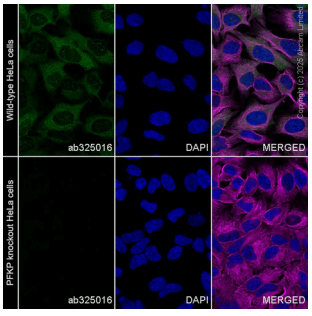



### Flow Cytometry (Intracellular) - Anti-PFKP antibody [EPR28830-157] (ab325016)

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

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized Parental HeLa (EDWT03) (Green) / PFKP KO HeLa (ED030126) (Magenta) cells labelling PFKP with ab325016 at 1/500 dilution (0.1ug) (Magenta) and Green compared with a Rabbit monoclonal IgG (ab172730) (Black) and Grey isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody).

Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/5000 dilution was used as the secondary antibody.




### Immunocytochemistry/ Immunofluorescence - Anti-PFKP antibody [EPR28830-157] (ab325016)

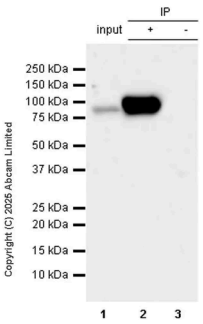
PFKP Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-PFKP antibody

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized PFKP KO HeLa (PFKP knockout human cervical adenocarcinoma epithelial cell))(ab265136) cells labelling PFKP with ab325016 at 1/200 (2.565 ug/ml) dilution, followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/ab150081 1000 2ug/ml dilution (Green).

Confocal image showing cytoplasmic and weak nuclear staining in parental HeLa cell line (shown in green). The counterstain was observed in magenta. Nuclear DNA was labelled with DAPI (shown in blue). Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

ab7291 Anti-alpha Tubulin mouse monoclonal antibody was used to counterstain tubulin at 1/ab7291 1000 1ug/ml dilution (Magenta). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/ab150081 1000 2ug/ml dilution.




## Immunoprecipitation - Anti-PFKP antibody [EPR28830-157] (ab325016)

PFKP was immunoprecipitated from 0.35 mg HeLa (human cervical adenocarcinoma epithelial cell) whole cell lysate with ab325016 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab325016 at 1/1000 dilution.

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

All lanes:
Immunoprecipitation - Anti-PFKP antibody [EPR28830-157] (ab325016) at 1/30 dilution

Lanes 1 - 2:
HeLa (human cervical adenocarcinoma epithelial cell) whole cell lysate at 10 µg

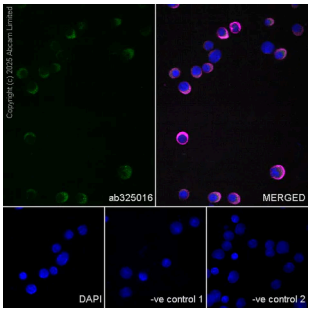
Lane 3:
Rabbit monoclonal IgG (ab172730) instead of ab325016 in HeLa whole cell lysate at 10 µg

Secondary

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

Observed band size: 85 kDa

Exposure time: 41s




## Immunocytochemistry/ Immunofluorescence - Anti-PFKP antibody [EPR28830-157] (ab325016)

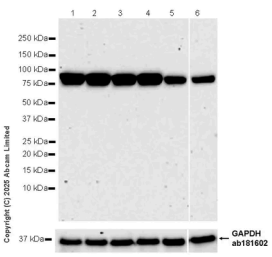
PFKP Immunocytochemistry/ Immunofluorescence staining of P815 (mouse mastocytoma mast cell) using rabbit Anti-PFKP antibody

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized P815 (mouse mastocytoma mast cell) cells labelling PFKP with ab325016 at 1/50 (10.26 ug/ml) dilution, followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/ab150081 1000 2ug/ml dilution (Green).

Confocal image showing cytoplasmic staining in P815 cell line (shown in green). The counterstain was observed in magenta. Nuclear DNA was labelled with DAPI (shown in blue). Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

ab7291 Anti-alpha Tubulin mouse monoclonal antibody was used to counterstain tubulin at 1/ab7291 1000 1ug/ml dilution (Magenta). The Nuclear counterstain was DAPI (Blue).

-ve control 1: ab325016 at 1/50 dilution, followed by ab150120 Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) antibody at 1/1000 dilution.
-ve control 2: ab7291 at 1/1000 dilution, followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) antibody at 1/1000 dilution.




## Western blot - Anti-PFKP antibody [EPR28830-157] (ab325016)

PFKP Western blot staining using rabbit Anti-PFKP antibody

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

In Western blot, Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602) staining at 1/200000 dilution.

All lanes:
Western blot - Anti-PFKP antibody [EPR28830-157] (ab325016) at 1/1000 dilution

Lane 1:
HeLa (human cervical adenocarcinoma epithelial cell) whole cell lysate at 20 µg

Lane 2:
Jurkat (human T cell leukemia T lymphocyte from peripheral blood) whole cell lysat at 20 µg

Lane 3:
MCF7 (human breast adenocarcinoma epithelial cell) whole cell lysate at 20 µg

Lane 4:
DU 145 (human prostate carcinoma epithelial cell) whole cell lysate at 20 µg

Lane 5:
Raji (human Burkitt's lymphoma B lymphocyte) whole cell lysate at 20 µg

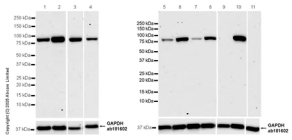
Lane 6:
PC-12 (rat adrenal gland pheochromocytoma cell) whole cell lysate at 20 µg

Secondary

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

Observed band size: 85 kDa, 36 kDa

Exposure time: 180s



## Western blot - Anti-PFKP antibody [EPR28830-157] (ab325016)

PFKP Western blot staining using rabbit Anti-PFKP antibody

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

Low expression: liver

Lanes 1-2 are incubated with are incubated with Goat Anti-Rabbit IgG (HRP) with minimal cross-reactivity with human IgG at 1/2000 and lanes 3-11 are incubated with Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (ab97051) at 1/20000.

In Western blot, Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602) staining at 1/200000 dilution.

All lanes:
Western blot - Anti-PFKP antibody [EPR28830-157] (ab325016) at 1/1000 dilution

Lane 1:
Human colon cancer tissue lysate at 20 µg

Lane 2:

Human lung cancer tissue lysate at 20 µg

Lane 3:
Mouse testis tissue lysate at 20 µg

Lane 4:
Rat testis tissue lysate at 20 µg

Lane 5:
Neuro-2a (mouse neuroblastoma neuroblast) whole cell lysate at 20 µg

Lane 6:
P815 (mouse mastocytoma mast Cell ) whole cell lysate at 20 µg

Lane 7:
F9 (mouse embryonal carcinoma epithelial cell) whole cell lysate at 20 µg

Lane 8:
Mouse brain tissue lysate at 20 µg

Lane 9:
Mouse liver tissue lysate at 20 µg

Lane 10:
Rat brain tissue lysate at 20 µg

Lane 11:
Rat liver tissue lysate at 20 µg

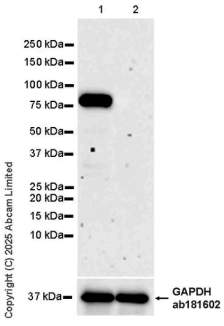
Secondary

Lanes 1 - 2:
Goat Anti-Rabbit IgG (HRP) with minimal cross-reactivity with human IgG at 1/2000 dilution

Lanes 3 - 11:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Observed band size: 85 kDa, 36 kDa

Exposure time: 180s




## Western blot - Anti-PFKP antibody [EPR28830-157] (ab325016)

PFKP Western blot staining using rabbit Anti-PFKP antibody

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

Performed under reducing conditions.

In Western blot, ab325016 was shown to bind specifically to PFKP. Target of interest was observed at 85 kDa in wild-type HeLa cell lysates (lane 1) with no signal observed at this size in PFKP knockout cell line (lane 2) (lane 2, knockout cell line ab265136/ knockout cell lysate ab257580).

In Western blot, Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602) staining at 1/200000 dilution.

All lanes:
Western blot - Anti-PFKP antibody [EPR28830-157] (ab325016) at 1/1000 dilution

Lane 1:
Wild type HeLa (human cervical adenocarcinoma epithelial cell) whole cell lysate at 20 µg

Lane 2:
PFKP knockout HeLa whole cell lysate at 20 µg

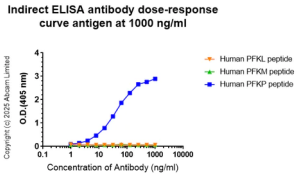
Secondary

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

Performed under reducing conditions.

Observed band size: 85 kDa, 36 kDa

Exposure time: 180s




## Indirect ELISA - Anti-PFKP antibody [EPR28830-157] (ab325016)

Indirect ELISA analysis of ab325016 at 1000-0 ng/ml. The Secondary antibody used was Alkaline Phosphatase-conjugated AffiniPure Goat Anti-Rabbit IgG (H+L) at 1:2500 dilution dilution.

Antigen: Human PFKP peptide, Human PFKM peptide, Human PFKL peptide.

Antigen concentration: 1000 ng/ml

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