PFKP (Phospho-Ser386) Antibody

#58002

Number: 58002-1, 58002-2

Amount: 50μg/50μl, 100μg/100μl

Swiss-Prot No. :Q01813

Form of Antibody: Rabbit IgG in phosphate buffered saline (without Mg2+ and Ca2+), pH 7.4, 150mM NaCl,0.02% sodium azide and 50% glycerol.

Storage/Stability: Store at -20℃/1 year

Immunogen: The antiserum was produced against synthesized phosphopeptide derived from Human PFKP around the phosphorylation site of serine 386 .

Purification: The antibody was affinity-purified from rabbit antiserum by affinity-chromatography using epitope-specific phosphopeptide. The antibody against non-phosphopeptide was removed by chromatography using non-phosphopeptide corresponding to the phosphorylation site.

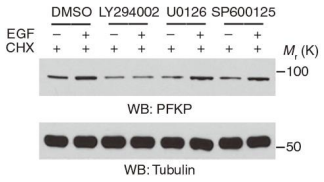
Specificity/Sensitivity:PFKP (phospho-Ser386) antibody detects endogenous levels of PFKP only when phosphorylated at serine 386.

Reactivity: Human,Mouse

Applications:

Predicted MW: 86kd

WB **:**1:500~1:2000



Serum-starved U251 cells were pretreated with CHX (100 μg ml^{-1}) for 1 h and then stimulated with EGF (100 ng ml^{-1}) for 12 h in the presence or absence of the indicated inhibitors.

Background :

PFK1 platelet isoform (PFKP) is the predominant PFK1 isoform in human glioblastoma cells and its expression correlates with total PFK activity. PFKP is overexpressed in human glioblastoma specimens due to an increased stability, which is induced by AKT activation resulting from phosphatase and tensin homologue (PTEN) loss and EGFR-dependent PI3K activation. AKT binds to and phosphorylates PFKP at S386, and this phosphorylation inhibits the binding of TRIM21 E3 ligase to PFKP and the subsequent TRIM21-mediated polyubiquitylation and degradation of PFKP. PFKP S386 phosphorylation increases PFKP expression and promotes aerobic glycolysis,

cell proliferation, and brain tumor growth. In addition, S386 phosphorylation in human glioblastoma specimens positively correlates with PFKP expression.

Application in thisArticle

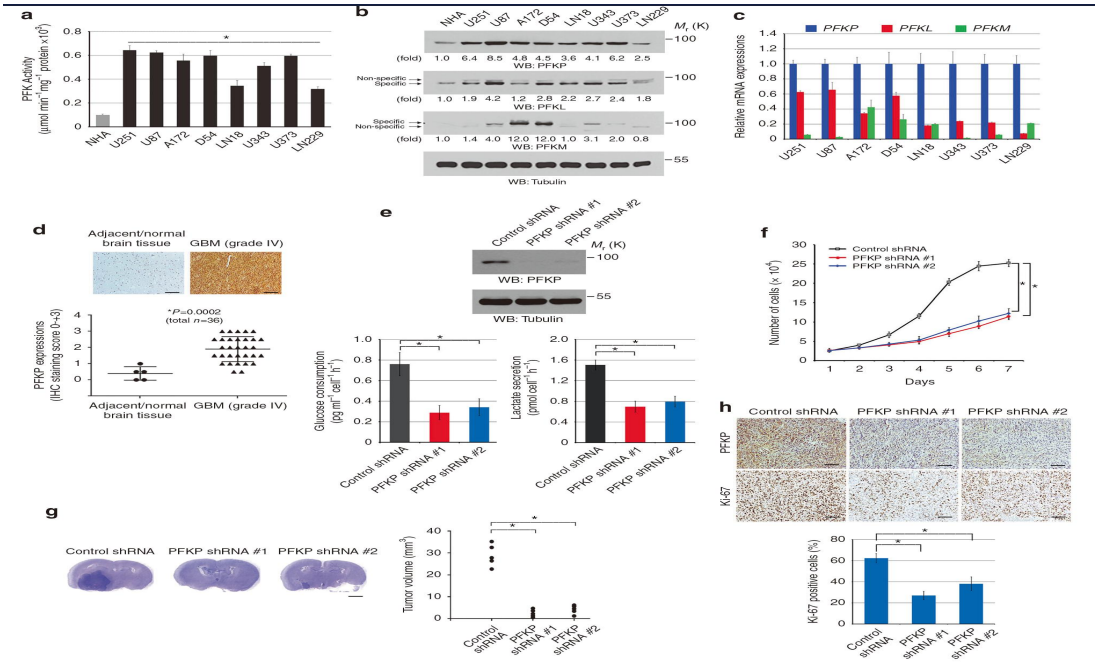


Fig. 1 PFKP expression is required for the Warburg effect and brain tumor growth. **a** PFK enzymatic activity was measured in normal human astrocytes (NHA) and the indicated GBM cells. Data represent the means ± s.d. of three independent experiments. *$P < 0.001$, based on the Student's *t* test. **b** The protein expression levels of PFK1 isoforms in NHA and the indicated GBM cells were determined by immunoblotting analyses with the indicated antibodies, respectively. **c** Relative mRNA expression levels of PFK1 isoforms were determined. The results were based on those in Supplementary Fig. 1a, c. **d** Microarrays of human GBM and normal brain tissue were immunostained with an anti-PFKP antibody. Representative images are shown (top panel). Data represent the means ± s.d. (bottom panel). *$P < 0.001$, based on the Student's *t* test. Scale bar, 100 μm. **e** U87/EGFRvIII cells were transfected with different shRNAs against PFKP. PFKP shRNA#1 was used for the subsequent experiments (top panel). The cells were cultured in no-serum DMEM for 24 h. The media were collected to analyze glucose consumption (bottom left panel) and lactate secretion (bottom right panel). All results were normalized to the final cell number. Data represent the means ± s.d. of three independent experiments. *$P < 0.001$, based on the Student's *t* test. **f** Control U87/EGFRvIII cells or PFKP-depleted U87/EGFRvIII cells were cultured in 1% serum medium for the indicated periods of time and harvested for cell counting. Data represent the mean ± s.d. of three independent experiments. *$P < 0.001$, based on the Student's *t* test. **g** A total of 5×10^5 control U87/EGFRvIII cells or PFKP-depleted U87/EGFRvIII cells were intracranially injected into athymic nude mice. After 2 weeks, the mice were euthanized and examined for tumor growth. Hematoxylin-and-eosin-stained coronal brain sections show representative tumor xenografts (left panel). Tumor volumes were measured by using length (*a*) and width (*b*) and calculated using the equation $V = ab^2/2$. Data represent the means ± s.d. of 5 mice (right panel). Note that the scores of some samples overlap. *$P < 0.001$, based on the Student's *t*-test. Scale bar, 2 mm. **h** IHC analyses of the tumor tissues were performed with anti-PFKP and anti-Ki-67 antibodies. Representative staining (top panel) and quantification of the staining (bottom panel) are shown. *$P < 0.001$, based on the Student's *t*-test. Scale bar, 100 μm

References:

Stabilization of phosphofructokinase 1 platelet isoform by AKT promotes tumorigenesis

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