Phosphofructokinase

Microplate Assay Kit

Cat #: orb390781 (manual)

Detection and Quantification of Phosphofructokinase (PFK) Activity in Urine, Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

For research use only. Not for diagnostic or therapeutic procedures.

Biorbyt Ltd
7 Signet Court, Swann Road, Cambridge, CB5 8LA. United Kingdom
Email: info@biorbyt.com | Phone: +44 (0)1223 859-353 | Fax: +44 (0)1223 280-240

Biorbyt LLC
68 TW Alexander Drive, Durham, NC 27709. United States
Email: info@biorbyt.com | Phone: +1 (415) 906-5211 | Fax: +1 (415) 651-8558

INTRODUCTION

Phosphofructokinase (PFK) is a key glycolytic enzyme and plays a major regulatory role during glycolysis. This enzyme is present in bacteria, plants and animals. There are 2 types of PFKs - PFK1 and PFK2. In the presence of ATP, PFK1 & PFK2 catalyzes the conversion of fructose-6-phosphate to fructose-1, 6-diphosphate and fructose-2, 6-diphosphate respectively and ADP. PFK has 3 major isoforms in mammals: PFK-M (muscle), PFK-L (liver) and PFK-P (platelet). In humans, PFK deficiency causes glycogen storage disease, also called Tarui's disease, which is characterized by exercise-induced muscle weakness and cramps. On the other hand, increased PFK activity contributes to cancer cell proliferation and tumorigenicity. Early detection of abnormal phosphofructokinase activity is crucial for diagnosis, prediction and therapeutic strategy. The Phosphofructokinase Microplate Assay Kit, PFK converts fructose-6-phosphate and ATP to fructose-diphosphate and ADP. NADH can be measured at a colorimetric readout at 340 nm.

KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	20 ml x 1	4 °C
Substrate	Powder x 1	-20 °C
Enzyme I	Powder x 1	-20 °C
Enzyme II	Powder x 1	-20 °C
Standard	Powder x 1	-20 °C
Technical Manual	1 Manual	

Note:

Substrate: add 17 ml Reaction Buffer to dissolve before use.

Enzyme I: add 1 ml distilled water to dissolve before use, store at 4 °C.

Enzyme II: add 1 ml distilled water to dissolve before use, store at 4 °C.

Standard: add 1 ml distilled water to dissolve before use; then add 0.2 ml into 0.8 ml distilled water, the concentration will be 400 μmol/L.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 340 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times) ; centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For serum or plasma samples

Detect directly.

ASSAY PROCEDURE

Warm all reagents to room temperature before use.

Add following reagents in the microplate:

Reagent	Sample	Standard	Blank
Standard	--	200 µl	--
Distilled water	--	--	200 µl
Sample	10 µl	--	--
Substrate	170 µl	--	--
Enzyme I	10 µl	--	--
Enzyme II	10 µl	--	--
Mix, measured at 340 nm and record the absorbance of 10th second and 130th second.			

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

CALCULATION

Unit Definition: One Unit of PFK activity is defined as the enzyme reduces 1 nmol of NADH per minute.

1. According to the protein concentration of sample

$$PFK\ (U/mg) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample\ (10S)} - OD_{Sample\ (130S))} / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein}) / T$$

$$= 4000 \times (OD_{Sample\ (10S)} - OD_{Sample\ (130S))} / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

$$PFK\ (U/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample\ (10S)} - OD_{Sample\ (130S))} / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times W / V_{Assay}) / T$$

$$= 4000 \times (OD_{Sample\ (10S)} - OD_{Sample\ (130S))} / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the quantity of cells or bacteria

$$PFK\ (U/10^4) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample\ (10S)} - OD_{Sample\ (130S))} / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times N / V_{Assay}) / T$$

$$= 4000 \times (OD_{Sample\ (10S)} - OD_{Sample\ (130S))} / (OD_{Standard} - OD_{Blank}) / N$$

4. According to the volume of sample

$$PFK\ (U/ml) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample\ (10S)} - OD_{Sample\ (130S))} / (OD_{Standard} - OD_{Blank}) / V_{Sample} / T$$

$$= 4000 \times (OD_{Sample\ (10S)} - OD_{Sample\ (130S))} / (OD_{Standard} - OD_{Blank})$$

$C_{Standard}$: the standard concentration, 400 μmol/L = 400 nmol/ml;

$V_{Standard}$: the volume of standard, 200 μl = 0.2 ml;

$C_{Protein}$: the protein concentration, mg/ml;

W: the weight of sample, g;

N: the quantity of cell or bacteria, N × 10^4;

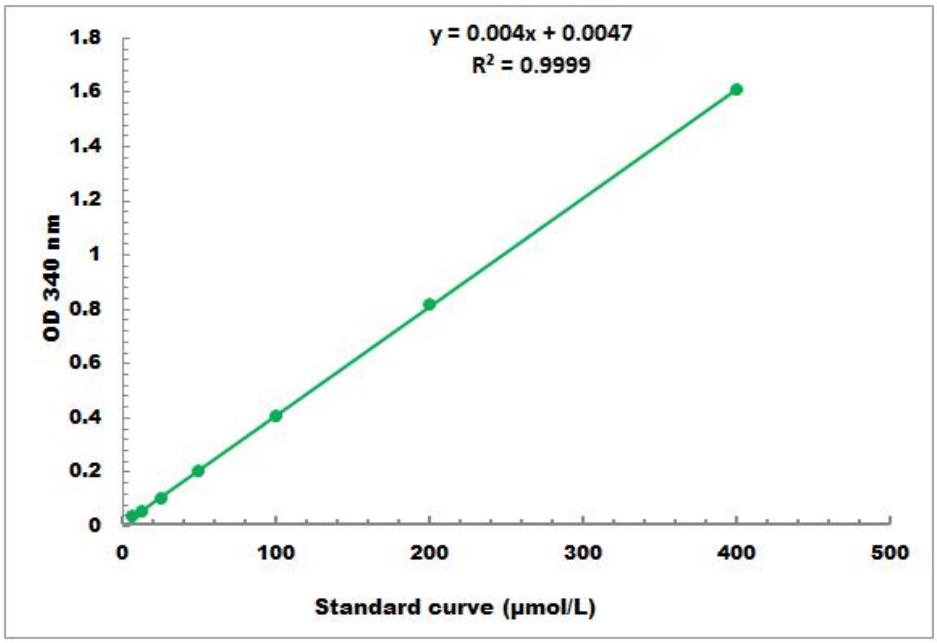
V_{Sample}: the volume of sample, 0.01 ml;

V_{Assay}: the volume of Assay buffer, 1 ml;

T: the reaction time, 2 minutes.

TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 4 µmol/L - 400 µmol/L

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