



# **Phosphofructokinase Microplate Assay Kit User Manual**

**Catalog # CAK1075**

(Version 1.4D)

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.**

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## I. 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.

## II. 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.

## III. 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

#### IV. 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  $5 \times 10^6$  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.

## V. ASSAY PROCEDURE

Warm all reagents to room temperature before use.

Add following reagents in the microplate:

Reagent	Sample	Standard	Blank
Standard	--	200 $\mu$ l	--
Distilled water	--	--	200 $\mu$ l
Sample	10 $\mu$ l	--	--
Substrate	170 $\mu$ l	--	--
Enzyme I	10 $\mu$ l	--	--
Enzyme II	10 $\mu$ l	--	--
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.
- 3) Reagents must be added step by step, can not be mixed and added together.

## VI. CALCULATION

**Unit Definition:** One Unit of PFK activity is defined as the enzyme reduces 1  $\mu\text{mol}$  of NADH per minute.

### 1. According to the protein concentration of sample

$$\begin{aligned} \text{PFK (U/mg)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}(10\text{S})} - \text{OD}_{\text{Sample}(130\text{S})}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / \\ &\quad (V_{\text{Sample}} \times C_{\text{Protein}}) / T \\ &= 4 \times (\text{OD}_{\text{Sample}(10\text{S})} - \text{OD}_{\text{Sample}(130\text{S})}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / C_{\text{Protein}} \end{aligned}$$

### 2. According to the weight of sample

$$\begin{aligned} \text{PFK (U/g)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}(10\text{S})} - \text{OD}_{\text{Sample}(130\text{S})}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / \\ &\quad (V_{\text{Sample}} \times W / V_{\text{Assay}}) / T \\ &= 4 \times (\text{OD}_{\text{Sample}(10\text{S})} - \text{OD}_{\text{Sample}(130\text{S})}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / W \end{aligned}$$

### 3. According to the quantity of cells or bacteria

$$\begin{aligned} \text{PFK (U}/10^4) &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}(10\text{S})} - \text{OD}_{\text{Sample}(130\text{S})}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / \\ &\quad (V_{\text{Sample}} \times N / V_{\text{Assay}}) / T \\ &= 4 \times (\text{OD}_{\text{Sample}(10\text{S})} - \text{OD}_{\text{Sample}(130\text{S})}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / N \end{aligned}$$

### 4. According to the volume of sample

$$\begin{aligned} \text{PFK (U/ml)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}(10\text{S})} - \text{OD}_{\text{Sample}(130\text{S})}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / \\ &\quad V_{\text{Sample}} / T \\ &= 4 \times (\text{OD}_{\text{Sample}(10\text{S})} - \text{OD}_{\text{Sample}(130\text{S})}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \end{aligned}$$

$C_{\text{Standard}}$ : the standard concentration, 400  $\mu\text{mol/L}$  = 0.4  $\mu\text{mol/ml}$ ;

$V_{\text{Standard}}$ : the volume of standard, 200  $\mu\text{l}$  = 0.2 ml;

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

W: the weight of sample, g;

N: the quantity of cell or bacteria,  $N \times 10^4$ ;

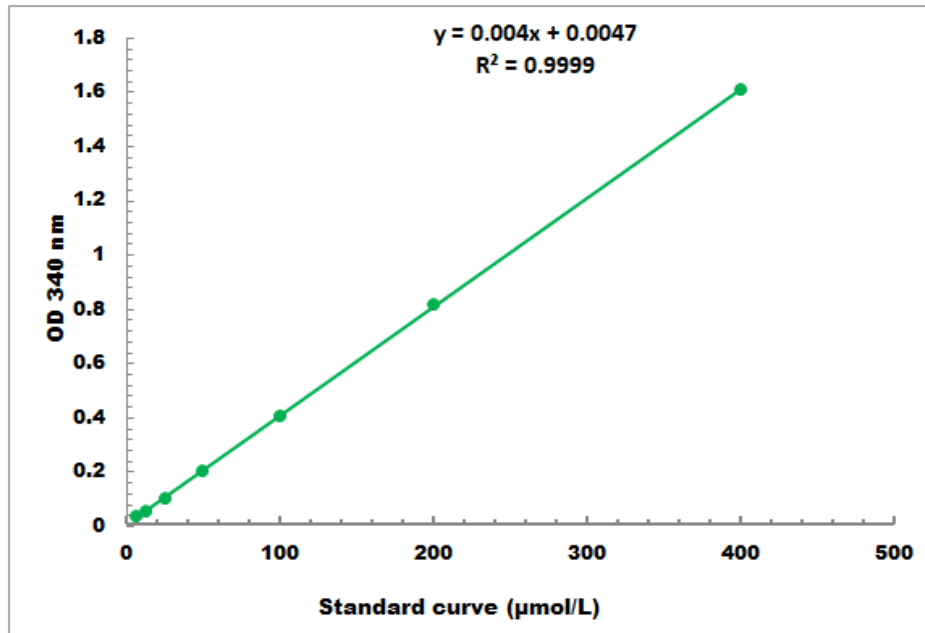
$V_{\text{Sample}}$ : the volume of sample, 0.01 ml;

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

T: the reaction time, 2 minutes.

## VII. 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

## VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to [www.cohesionbio.com](http://www.cohesionbio.com) or contact us at [techsupport@cohesionbio.com](mailto:techsupport@cohesionbio.com)

## IX. NOTES