Total Phosphatase

Microplate Assay Kit

User Manual

Catalog # CAK1193

(Version 1.3B)

Detection and Quantification of Total Phosphatase 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.

I. INTRODUCTION

Para-nitrophenyl phosphate (pNPP) is a chromogenic substrate for most phosphatases such as alkaline phosphatases, acid phosphatases, protein tyrosine phosphatases and serine/threonine phosphatases.

Total Phosphatase Microplate Assay Kit provides a simple and direct procedure for measuring phosphatase activity in a variety of samples. The reaction yields para-nitrophenol, which becomes an intense yellow soluble product under alkaline conditions and can be conveniently measured at 405 nm on a spectrophotometer.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Substrate	Powder x 1	4 °C
Stop Solution	10 ml x 1	4 °C
Standard (500 µmol/L)	1 ml x 1	4 °C
Positive Control	Powder x 1	-20 °C
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Note:

Substrate: add 9 ml distilled water to dissolve before use.

Positive Control: add 0.2 ml distilled water to dissolve before use.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 405 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Centrifuge
6. Timer

IV. SAMPLE PREPARATION

1. For liquid samples

Serially dilute sample in a proper Enzyme Buffer (not provide), then detect directly.

V. ASSAY PROCEDURE

Equilibrate all reagents to room temperature by allowing them to stand for 30 minutes at room temperature.

Add following reagents into the microplate:

Reagent	Sample	Control	Standard	Blank	Positive Control
Substrate	90 µl	90 µl	--	--	90 µl
Standard	--	--	100 µl	--	--
Sample	10 µl	--	--	--	--
Positive Control	--	--	--	--	10 µl
Distilled water	--	10 µl	--	100 µl	--
Incubate at room temperature for 10 minutes.					
Stop Solution	100 µl	100 µl	100 µl	100 µl	100 µl
Mix, read the absorbance measured at 405 nm.					

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 Phosphatase activity is defined as the enzyme generates 1 μmol p-nitrophenol per minute.

1. According to the protein concentration of sample

$$\text{Phosphatase (U/mg)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} / C_{Protein} / T$$

$$= 0.5 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

3. According to the volume of sample

$$\text{Phosphatase (U/ml)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} / T$$

$$= 0.5 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank})$$

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

$C_{Standard}$: the concentration of standard, 500 μmol/L = 0.5 μmol/ml;

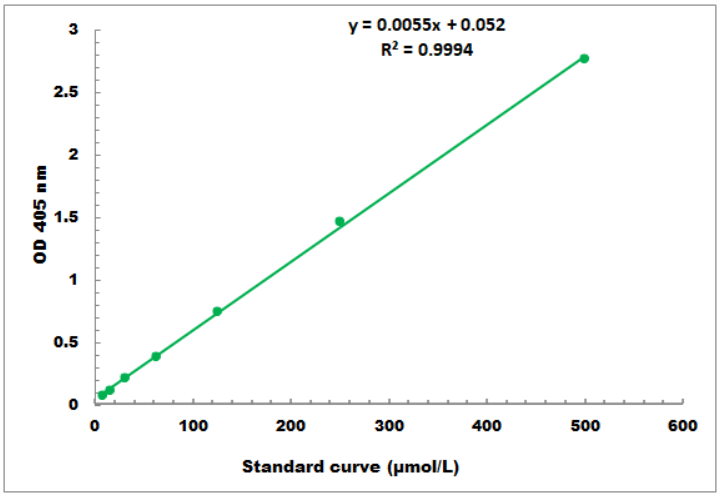
$V_{Standard}$: the total volume of standard, 0.1 ml;

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

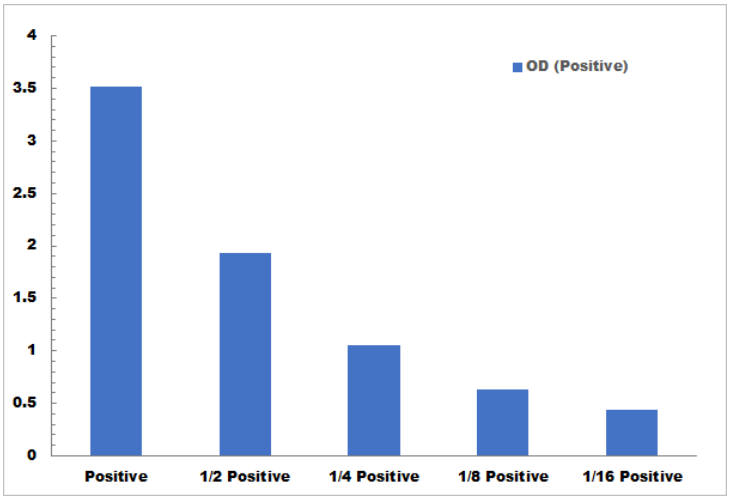
T: the reaction time, 10 minutes.

VII. TYPICAL DATA

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



Detection Range: 5 μmol/L - 500 μmol/L



Positive Control reaction in 96-well plate assay with decreasing the concentration

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES