Total Phosphatase Activity

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1193

(Version 1.4B)

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 Activity Colorimetric Microplate Assay Kitprovides 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	Powderx 1	4 °C
Stop Solution	10 mlx 1	4 °C
Standard (500µmol/L)	1 mlx 1	4 °C
Positive Control	Powder x 1	-20 °C
Technical Manual	1 Manual	

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. REAGENT PREPARATION

Standard:Briefly centrifuge prior to opening.Perform 2-fold serial dilutions of the top standardsolution using distilled waterto make the standard curve. The concentration of standard curve could be 500/250/125/62.5/31.2/15.6/7.8µmol/L.

Substrate: Add 9 ml distilled water to dissolve before use.Keep in dark and store at 4 °C for 3-4 weeks or -20°C for 3months after reconstitution.

Positive Control: Briefly centrifuge prior to opening. Add 0.2 ml distilled waterto dissolve before use.Store at -80°C for 1 month after reconstitution.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. SAMPLE PREPARATION

1.For liquid samples

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

VI. ASSAY PROCEDURE

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

Add following reagents intothe microplate:

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

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**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 samples into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

Unit Definition: One unit of Phosphatase activity is defined as the enzyme generates 1 μmolp-nitrophenol per minute.

1. According to the slope of the standard curve

$$\text{Activity} = \frac{(OD_{Sample}-OD_{Control}) - \text{Intercept}}{\text{Slope}\times T} \times \frac{V_{Standard}}{V_{Sample}} \times n(U/mL)$$

2. According to one point of the standard OD value and concentration

2.1 According to the protein concentration of sample

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

2.2 According to the volume of sample

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

Slope: the absorbance slope of standard curve

n: the dilution factor

$C_{Protein}$: the protein concentration of sample, mg/mL

$C_{Standard}$: the concentration of standard, μmol/mL

$V_{Standard}$: the volume of standard in assay procedure, mL

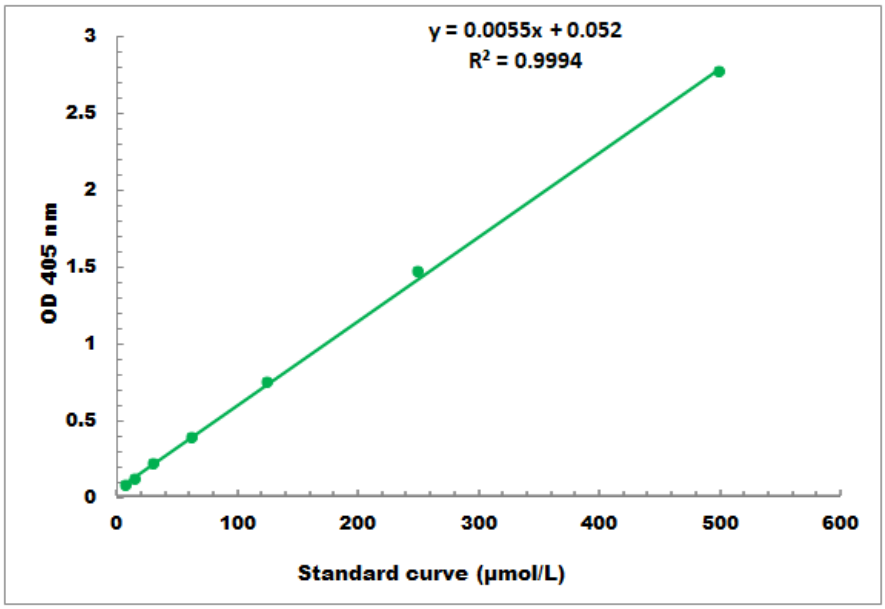
V_{Sample}: the volume of sample in assay procedure, mL

V_{Assay}: the volume of Assay Buffer in sample preparation, mL

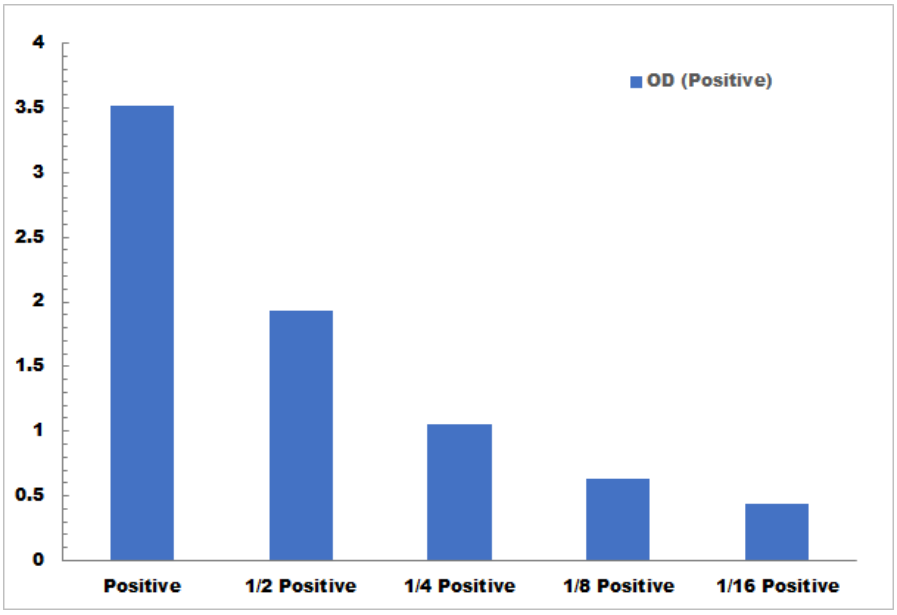
T: the reaction time, minute

VIII. TYPICAL DATA

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



Detection Range: 5μmol/L -500 μmol/L



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