



# **Neutral Phosphatase Microplate Assay Kit User Manual**

**Catalog # CAK1225**

(Version 1.3B)

Detection and Quantification of Neutral Phosphatase (NP) 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

Neutral Phosphatase dephosphorylate phosphate groups from phosphate esters under neutral conditions.

Neutral Phosphatase Microplate Assay Kit is a sensitive assay for determining neutral phosphatase activity in various samples. The assay is initiated with the enzymatic hydrolysis of the disodium phenyl phosphate by acid phosphatase. The enzyme catalysed reaction products can be measured at a colorimetric readout at 510 nm.

## II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	4 ml x 1	4 °C, keep in dark
Substrate	Powder x 1	4 °C, keep in dark
Dye Reagent I	Powder x 1	4 °C, keep in dark
Dye Reagent II	Powder x 1	4 °C, keep in dark
Standard	Powder x 1	4 °C
Positive Control	Powder x 1	-20 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

### Note:

**Substrate:** add 4 ml distilled water to dissolve before use.

**Dye Reagent I:** add 9 ml distilled water to dissolve before use.

**Dye Reagent II:** add 2 ml distilled water to dissolve before use.

**Standard:** add 1 ml distilled water to dissolve, then add 50 µl standard into 950 µl distilled water, the concentration will be 5 mmol/L.

**Positive Control:** add 0.2 ml assay buffer to dissolve before use, mix.

### **III. MATERIALS REQUIRED BUT NOT PROVIDED**

1. Microplate reader to read absorbance at 510 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Ice
7. Centrifuge
8. Timer

### **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, or dilute with Assay Buffer.

## V. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent	Sample	Standard	Blank	Positive Control
Sample	10 $\mu$ l	--	--	--
Standard	--	10 $\mu$ l	--	--
Distilled water	--	--	10 $\mu$ l	--
Positive Control	--	--	--	10 $\mu$ l
Reaction Buffer	40 $\mu$ l	40 $\mu$ l	40 $\mu$ l	40 $\mu$ l
Substrate	40 $\mu$ l	40 $\mu$ l	40 $\mu$ l	40 $\mu$ l
Mix, put it in the oven, 37 °C for 15 minutes.				
Dye Reagent I	90 $\mu$ l	90 $\mu$ l	90 $\mu$ l	90 $\mu$ l
Dye Reagent II	20 $\mu$ l	20 $\mu$ l	20 $\mu$ l	20 $\mu$ l
Mix, wait for 10 minutes, record absorbance measured at 510 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 Neutral Phosphatase activity is defined as the enzyme generates 1  $\mu\text{mol}$  phenol per minute.

1. According to the protein concentration of sample

$$\begin{aligned}\text{NP (U/mg)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / V_{\text{Sample}} / \\ &\quad C_{\text{Protein}} / T \\ &= 0.333 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / C_{\text{Protein}}\end{aligned}$$

2. According to the weight of sample

$$\begin{aligned}\text{NP (U/g)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (V_{\text{Sample}} \times \\ &\quad W / V_{\text{Assay}}) / T \\ &= 0.333 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / W\end{aligned}$$

3. According to the volume of serum or plasma

$$\begin{aligned}\text{NP (U/ml)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / V_{\text{Sample}} / T \\ &= 0.333 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}})\end{aligned}$$

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

W: the weight of sample, g;

$C_{\text{Standard}}$ : the concentration of standard, 5 mmol/L = 5  $\mu\text{mol/ml}$ ;

$V_{\text{Standard}}$ : the volume of standard, 0.01 ml;

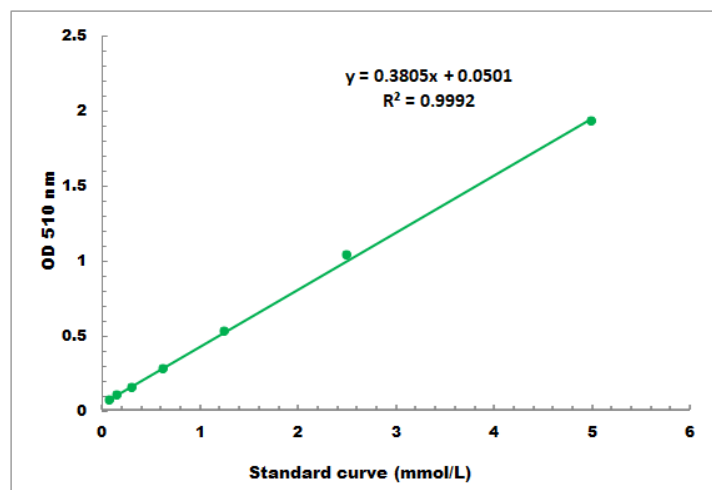
$V_{\text{Sample}}$ : the volume of sample, 0.01 ml;

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

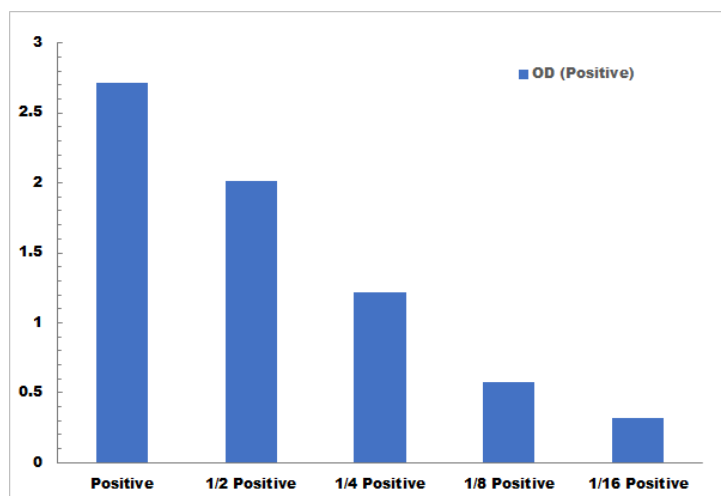
T: the reaction time, 15 minutes.

## VII. TYPICAL DATA

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



Detection Range: 0.05 mmol/L - 5 mmol/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](http://www.cohesionbio.com) or contact us at [techsupport@cohesionbio.com](mailto:techsupport@cohesionbio.com)

## IX. NOTES