Phosphorus

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1108

(Version 1.2C)

Detection and Quantification ofPhosphorusContentinSerum, Urine, Saliva, Tissue extracts and Other biological fluidsSamples.

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

I. INTRODUCTION

Phosphorus is an important part of several of your body's processes. It helps with bone growth, energy storage, and nerve and muscle production. Many foods, especially meats and dairy products, contain phosphorus, so it's usually easy to get enough of this mineral in your diet. Most of your body's phosphorus is contained in your bones and teeth. However, some in your blood. Hyperphosphatemia is when you have too much phosphorus in your blood. Hypophosphatemia is the opposite: having too little phosphorus. Various conditions, including liver disease and vitamin D deficiency, can cause your blood phosphorus level to become too high or too low.

Phosphorus Colorimetric Microplate Assay Kitprovides a sensitive colorimetric means to directly measurephosphorus concentration in various samples. Phosphorus concentrationis based on the reaction of phosphorus with ammonium molybdate toform a blue colored product. The color intensity at 620 nm is directly proportional to phosphorusconcentration in the sample.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 mlx 4	4 °C
Reaction Buffer	5 mlx 1	4 °C
Dye Reagent	Powderx 1	4 °C
Standard (0.4mmol/L)	1 mlx 1	4 °C
Technical Manual	1 Manual	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 620 nm
2. Distilled water
3. Pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer

IV. REAGENT PREPARATION

Dye Reagent: add 5 ml distilled waterto dissolve before use.Store at -20 °C for 1-2days.

V. SAMPLE PREPARATION

1.For liquidsamples

Add 100 µlsampleand 900 µlAssay Bufferinto the microcentrifuge tube, mix, centrifuged at 10,000rpm for 10 minutes, take the supernatant into a new centrifuge tube for detection.

2.For tissue samples

Weigh out 0.1 g tissue, homogenize with 500 µl distilled water in a centrifuge tube;then add 500 µl Assay Buffer, centrifuged at 10,000 rpm for 10minutes, take the supernatant into a new centrifuge tube for detection.

VI. ASSAY PROCEDURE

Add following reagents intothe microplate:

Reagent*	Blank	Standard	Sample**
Distilled water	100µl	--	--
Standard	--	100µl	--
Sample	--	--	100µl
Reaction Buffer	50 µl	50 µl	50 µl
Dye Reagent	50 µl	50 µl	50 µl
Mix, wait for 10 minutes, measured at 620 nm and recordthe absorbance.			

Note:

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

**The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

**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

1. Calculatethe sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

$$C=\frac{(OD_{Sample}-OD_{Blank})-\text{Intercept}}{\text{Slope}}\times n(mmol/L)$$

Calculate the initial concentration according to sample preparation procedure.

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

2.1. According to the volume of sample

$$C=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank})\times V_{Sample}}\times 10(\mu mol/ml)$$

2.2. According to the weight of sample

$$C=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank})\times(W\times V_{Sample}/V_{Assay})}(\mu mol/g)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

W: the weight of sample, g

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

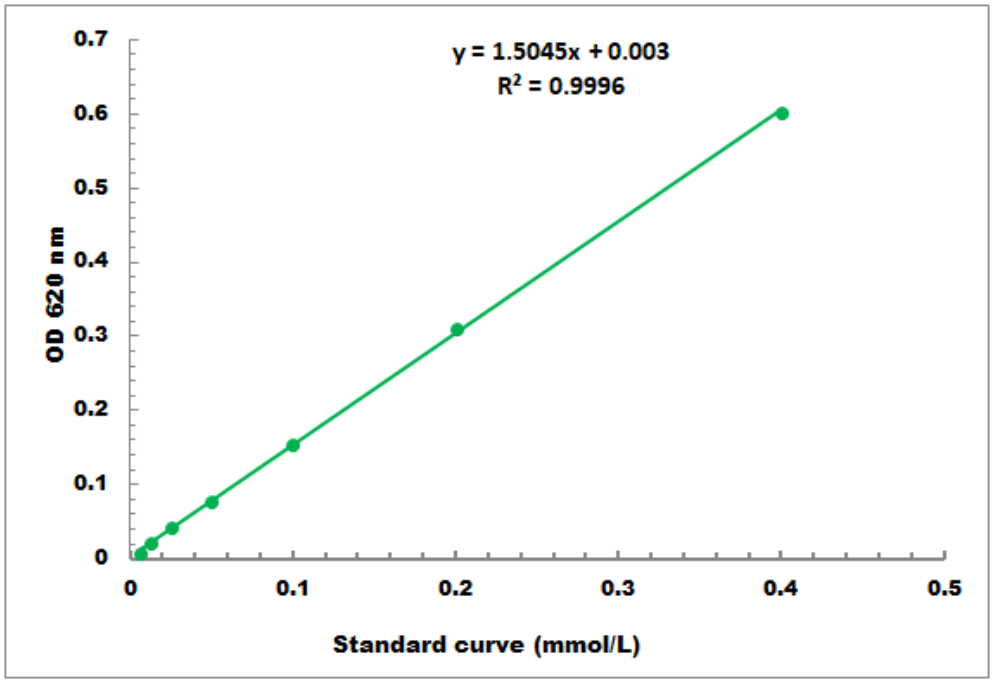
V_{Sample}: the volume of sample in assay procedure, mL

V_{Assay}: the volume of distilled water and Assay Buffer, mL

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

VIII. TYPICAL DATA

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



Detection Range: 0.01mmol/L-0.4mmol/L