PRODUCT INFORMATION & MANUAL

Inorganic Phosphorus Assay Kit (Colorimetric)

NBP3-25934

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

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Novus kits are guaranteed for 6 months from date of receipt

Phosphorus (Pi) Colorimetric Assay Kit
(Phospho Molybdate Method)

Catalog No: NBP3-25934

Method: Colorimetric method

Specification: 100 Assays (Can detect 96 samples without duplication)

Instrument: Spectrophotometer

Sensitivity: 0.005 mmol/L

Detection range: 0.005-2.0 mmol/L

Average intra-assay CV (%): 1.0

Average inter-assay CV (%): 1.3

Average recovery rate (%): 102

- ▲ This kit is for research use only.
- ▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
- ▲ Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

This kit can be used to measure phosphorus (Pi) content in serum, plasma, tissue and other samples.

▲Background

Phosphorus is an important mineral that maintains cellular energy, mineralizes bones, and protects non-bone tissue from calcification. Inorganic phosphorus is the component of DNA, RNA, ATP and phospholipid. Phosphorus is existed in the form of ester and phosphate anion in the whole blood. The concentration of phosphorus is strictly regulated by specific ion transport proteins and hormones.

▲Detection principle

Inorganic phosphorus react with molybdic acid to produce phosphomolybdic acid. Phosphomolybdic acid can be reduced to molybdenum blue under the action of reducing agent. And the molybdenum blue have a maximum absorption peak at 660 nm. The phosphorus content can be calculated indirectly be measuring the OD value at 660 nm.

▲Kit components & Storage

Item	Component	Specification	Storage
Reagent 1	Chromogenic Agent A	50 mL × 1 vial	2-8℃ , 12 months
Reagent 2	Chromogenic Agent B	Powder × 4 vials	2-8℃ , 12 months, shading light
Reagent 3	Chromogenic Agent C	Powder × 2 vials	2-8℃ , 12 months, shading light
Reagent 4	Protein Precipitator	60 mL × 1 vial	2-8℃ , 12 months
Reagent 5	10 mmol/L Phosphorus Standard	1 mL × 1 vial	2-8℃ , 12 months
Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other.			

▲Materials prepared by users

Instruments

Spectrophotometer (660 nm), Water bath, Centrifuge, Micropipettor, Vortex mixer

Reagents

Double distilled water, Normal saline (0.9% NaCl)

▲Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

▲Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

Pre-assay preparation

▲Reagent preparation

1. Preparation of reagent 2 working solution:
   dissolve a vial of powder with 12.5 mL double distilled water and mix fully. The prepared solution can be stored at 2-8℃ for 5 days.
2. Preparation of reagent 3 working solution:
   dissolve a vial of powder with 25 mL double distilled water and mix fully. The prepared solution can be stored at 2-8℃ for 2 months.
3. Preparation of chromogenic agent:
   Prepare the chromogenic agent according to the ratio of double distilled water: reagent 1: reagent 2 working solution: reagent 3 working solution =2: 1: 1: 1 (mix fully). Prepare the fresh solution before use.
4. Preparation of 0.5 mmol/L standard solution:
   Dilute the reagent 5 with double distilled water at a ratio of 1:19 and mix fully. Prepare the fresh solution before use.

▲Sample preparation

The samples should be prepared as conventional methods. Also please refer to appendix II.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the formal experiment and the detection range (0.005-2.0 mmol/L).

The recommended dilution factor for different samples is as follows (for reference only)

Sample type	Dilution factor
Human serum	1
Human plasma	1
10% Mouse kidney tissue homogenization	1
10% Mouse heart tissue homogenization	1
10% Mouse liver tissue homogenization	1

Note: The diluent is double distilled water .

Assay protocol

▲Detailed operating steps

1. The preparation of sample supernatant

Take 0.1 mL of serum (plasma) or 10% tissue homogenate sample, then add 0.4 mL of reagent 4, mix fully. Centrifuge at 1100 g for 10 min and take the supernatant for detection.

2. The preparation of sample supernatant

(1) Blank tube: Take 0.2 mL of double distilled water to an EP tube.
Standard tube: Take 0.2 mL of 0.5 mmol/L standard solution to an EP tube.
Sample tube: Take 0.2 mL of sample supernatant to the EP tubes.

(2) Add 2.0 mL of chromogenic agent into each tube of Step 1 and mix fully.

(3) Incubate the tubes at 37℃ for 30 min, then cool the tubes to room temperature.

(4) Set the spectrophotometer to zero with double distilled water, and measure the OD at 660 nm with 1 cm optical path quartz cuvette.

▲Summary operation table

	Blank tube	Standard tube	Sample tube
Double distilled water (mL)	0.2		
0.5 mmol/L standard solution (mL)		0.2	
Sample supernatant (mL)			0.2
Chromogenic agent (mL)	2.0	2.0	2.0
Mix fully, then incubate the tubes at 37℃ for 30 min, then cool the tubes to room temperature. Set the spectrophotometer to zero with double distilled water, and measure the OD at 660 nm.			

▲Calculation

For serum/plasma samples:

$$\underset{(\text{mmol/L})}{\text{Pi}} = \frac{\Delta A_1}{\Delta A_2} \times c \times 5 \times f$$

For tissue samples:

$$\underset{(\text{mmol/gprot})}{\text{Pi}} = \frac{\Delta A_1}{\Delta A_2} \times c \times 5 \times f \div C_{pr}$$

Note:

ΔA_1: OD_{sample}-OD_{blank}

ΔA_2: $OD_{standard}$-OD_{blank}

c: The concentration of standard (0.5 mmol/L).

5: Dilution factor of sample in preparation of supernatant.

f: Dilution factor of sample before tested.

C_{pr}: Concentration of protein in sample (gprot/L).

Appendix I Data

▲Example analysis

Take 0.1 mL of human serum, carry the assay according to the operation table. The results are as follows:

The average OD value of the sample is 0.396, the average OD value of the blank is 0.011, the average OD value of the standard is 0.373, and the calculation result is:

$$Pi(mmol/L)=[(0.396-0.011)\div(0.373-0.011)]\times0.5\times5\times1$$
$$=2.66\ (mmol/L)$$

Appendix II Sample preparation

The following sample pretreatment methods are for reference only.

▲ Serum

Collect fresh blood and stand at 25℃ for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4℃ . Take the serum (which is the upper light yellow clarified liquid layer) to preserve it on ice for detection. If not detected on the same day, the serum can be stored at -80℃ for a month.

▲ Plasma

Take fresh blood into the tube which has heparin anticoagulant, centrifuge at 700-1000 g for 10 min at 4℃ . Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) to preserve it on ice for detection. If not detected on the same day, the plasma can be stored at -80℃ for a month.

▲ Tissue sample

Take 0.02-1g fresh tissue to wash with homogenization medium at 2-8℃ . Absorb the water with filter paper and weigh. Homogenize at the ratio of the volume of homogenized medium (2-8℃) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 1500 g at 4℃ . Take the supernatant to preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M, E-BC-K168-S, E-BC-K165-S). If not detected on the same day, the tissue sample (without homogenization) can be stored at -80℃ for a month.

Note:

1. Homogenized medium: 0.9% NaCl.

2. Homogenized method:

   (1) Hand-operated: Weigh the tissue and mince to small pieces (1 mm^3), then put the tissues pieces to glass homogenized tube. Add homogenized medium into homogenized tube, place the tube into the ice bath with left hand, and insert the glass tamping rod vertically into the homogenized tube with the right hand to grind up and down for 6-8 min.

   Or put the tissue into the mortar, and add liquid nitrogen to grind fully. Then add the homogenized medium to homogenize.

   (2) Mechanical homogenate: Weigh the tissue to EP tube, add the homogenized medium to homogenize the tissue with homogenizer instrument (60 Hz, 90s) in the ice bath. (For samples of skin, muscle and plant tissue, the time of homogenization can be properly prolonged.)

Appendix III References

1. Kestenbaum B, Glazer N L, Kottgen A, et al. Common genetic variants associate with serum phosphorus concentration[J]. Journal of the American Society of Nephrology, 2010, 21(7): 1223-1232.

2. Dimeglio L A, White K E, Econs M J. Disorders of phosphate metabolism [J]. Endocrinology & Metabolism Clinics of North America, 2000, 29(3): 591-609.