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

Catalog No: E-BC-K245-M

Method: Colorimetric method

Specification: 96T (Can detect 80 samples without duplication)

Measuring instrument: Microplate reader

Sensitivity: 0.004 mmol/L

Detection range: 0.004-2.0 mmol/L

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, urine and tissue 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	20 mL×1 vial	2-8℃ , 6 months
Reagent 2	Chromogenic Agent B	Powder × 2 vials	2-8℃ , 6 months, shading light
Reagent 3	Chromogenic Agent C	Powder × 2 vials	2-8℃ , 6 months, shading light
Reagent 4	Protein Precipitator	40 mL × 1 vial	2-8℃ , 6 months
Reagent 5	10 mmol/L Phosphorus Standard	1 mL × 1 vial	2-8℃ , 6 months
	Microplate	96 wells	
	Plate Sealer	2 pieces	

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

Microplate reader (620-690 nm), Micropipettor, Incubator, Vortex mixer, Centrifuge

Consumptive material

Tips (10 μL, 200 μL, 1000 μL), EP tubes (1.5 mL, 2 mL, 5 mL)

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.

▲ The key points of the assay

1. Prevent the formulation of bubbles when adding the liquid to the microplate.
2. Chromogenic agent should be prepared freshly.
3. Avoid the contamination of phosphorus, it is recommended to use disposable test tubes.

Pre-assay preparation

▲ Reagent preparation

1. Preparation of reagent 2 working solution

   Dissolve a vial of reagent 2 with 10 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 reagent 3 with 10 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.

▲ 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 pre-experiment and the detection range (0.004-2.0 mmol/L).

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

Sample type	Dilution factor
Human serum	1
Mouse serum	1
Rat plasma	1
Human urine	2-3
10% Rat liver tissue homogenate	1
10% Mouse lung tissue homogenate	1
10% Mouse brain tissue homogenate	1
10% Rat muscle tissue homogenate	1

Note: The diluent is normal saline (0.9% NaCl).

Assay protocol	
Ambient temperature	25-30℃
Optimum detection wavelength	660nm

Instructions for the use of transferpettor:

(1) Equilibrate the pipette tip in that reagent before pipetting each reagent.

(2) Don't add the liquid outside the tips into the reaction system when pipetting each reagent.

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

[Note]：A - H, standard wells; S1 - S80, sample wells.

▲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 standard curve
   Dilute 10 mmol/L standard stock solution with normal saline to a serial concentration. The recommended dilution gradient is as follows: 0, 0.1, 0.2, 0.5, 0.8, 1, 1.5, 2 mmol/L.

3. The measurement of samples

1) Standard well: Take 35 μL of standard solution with different concentration to the well.

Sample well: Take 35 μL of sample supernatant to the corresponding well.

2) Add 200 μL of chromogenic agent to each well and mix fully.

3) Mix fully with microplate reader for 10 s and incubate at 37℃ for 30 min.

4) Measure the OD value of each well at 660 nm with microplate reader.

▲Operation table

	Standard well	Sample well
Standard solution with different concentration (μL)	35	
Sample (μL)		35
Chromogenic agent (μL)	200	200
Mix fully with microplate reader for 10 s and incubate at 37℃ for 30 min. Measure the OD value of each well at 660 nm with microplate reader.		

▲Calculation

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample. The standard curve is: $y = ax + b$.

1. For serum/plasma samples

$$\text{Pi (mmol/L)} = (\Delta A_{660} - b) \div a \times 5 \times f$$

2. For tissue samples

$$\underset{(\text{mmol/gprot})}{\text{Pi}} = (\Delta A_{660} - b) \div a \times 5 \times f \div C_{pr}$$

Note:

y: $OD_{Standard} - OD_{Blank}$.($OD_{Blank}$ is the OD value when the standard concentration is 0).

x: The concentration of standard.

a: The slope of standard curve.

b: The intercept of standard curve.

f: Dilution factor of sample before test.

ΔA_{660}: $OD_{Sample} - OD_{Blank}$.

Cpr: Concentration of protein in sample (gprot/L).

5: Dilution factor of sample in preparation of supernatant.

▲Notes

1. This kit is for research use only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 6 months.
4. Do not use components from different batches of kit.

Appendix I Performance characteristics

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Detection range	0.004-2.0 mmol/L	Average intra-assay CV (%)	2.1
Sensitivity	0.004 mmol/L	Average inter-assay CV (%)	3.0
Average recovery rate (%)	101		

▲Example analysis

Take 100 μL of human serum sample and carry the assay according to the operation table. The results are as follows:

Standard curve: y = 0.9268 x + 0.0059, the average OD value of the sample is 0.652, the average OD value of the blank is 0.062, and the calculation result is:

$$\text{Pi (mmol/L)} = \frac{0.652 - 0.062 - 0.0059}{0.9268} \times 5 = 3.15\text{mmol/L}$$

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 anticoagulant (Heparin is used as 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.

▲ Urine

Collect fresh urine and centrifuge at 10000 g for 15 min at 4℃ . Take the supernatant to preserve it on ice for detection. If not detected on the same day, the urine can be stored at -80℃ for a month.

▲ Tissue sample

Take 0.02-1g fresh tissue to wash with normal saline (0.9% NaCl) 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 10000 g at 4℃ . Take the supernatant to preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80℃ for a month.

Note:

1. Homogenized medium: Normal saline (0.9% NaCl) or Double distilled water.
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.)

▲ Notes for sample

1. Please predict the concentration before assaying. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
2. If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.
3. If a lysis buffer is used to prepare tissue homogenates, there is a possibility of causing a deviation due to the introduced chemical substance.