Technical Manual

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

- Catalogue Code: MAES0161
- Size: 96T
- Research Use Only

1. Key features and Sample Types

Detection method:

Colorimetric method

Specification:

96T

Range:

0.004-2.0 mmol/L

Sensitivity:

0.004 mmol/L

Storage:

2-8°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

2. 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.

3. Intended Use

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

4. 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.

5. Kit components & storage

Item	Specification	Storage
Chromogenic Agent A	20 mL×1 vial	2-8°C, 6 months
Chromogenic Agent B	Lyophilized × 2 vials	2-8°C, 6 months, avoid direct sunlight
Chromogenic Agent C	Lyophilized × 2 vials	2-8°C, 6 months, avoid direct sunlight
Protein Precipitant	40 mL × 1 vial	2-8°C, 6 months
Standard Stock Solution (10 mmol/L)	1 mL × 1 vial	2-8°C, 6 months
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Microplate Reader (620-690 nm)
- Tips (10 µL, 200 µL, 1000 µL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- Normal Saline (0.9% NaCl)

6. Assay Notes:

1. Prevent the formation of bubbles when adding the liquid to the microplate.

2. Chromogenic agent should be prepared fresh.

3. Prevent the contamination of phosphorus, it is recommended to use disposable test tubes.

7. Reagent preparation:

1. Bring all reagents to room temperature before use.

2. Preparation of **chromogenic agent working solution B:** Dissolve a vial of chromogenic agent B with 10 mL double distilled water and mix fully. The prepared solution can be stored at 2-8°C for 5 days.

3. Preparation of **chromogenic agent working solution C:** Dissolve a vial of chromogenic agent C with 10 mL double distilled water and mix fully. The prepared solution can be stored at 2-8°C for 2 months.

4. Preparation of **chromogenic agent:** Prepare the chromogenic agent according to the ratio of double distilled water: chromogenic agent A: chromogenic agent working solution B: chromogenic agent working solution C =2: 1: 1: 1 (mix fully). Prepare the fresh solution before use.

8. Sample Preparation

1. Serum sample:
Collect fresh blood and stand at 25°C for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4°C. 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°C for a month.

2. Plasma sample:
Take fresh blood into the tube which has anticoagulant (Heparin is used as anticoagulant), centrifuge at 700-1000 g for 10 min at 4°C. 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°C for a month.

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

4. Tissue sample:

Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8°C. Absorb the water with filter paper and weigh. Homogenize at the ratio of the volume of Normal saline (0.9% NaCl) or Double distilled water (2-8°C) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4°C. Take the supernatant to preserve it on ice for detection. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80°C for a month.

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. 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);

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 660 nm

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.

10. Operation Steps

The preparation of standard curve

Dilute standard stock solution (10 mmol/L) 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.

The preparation of sample supernatant

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

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°C 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°C for 30 min. Measure the OD value of each well at 660 nm with microplate reader.		

11. Calculations

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. Serum (plasma) sample:

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

2. Tissue sample:

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

y: $OD_{Standard} - OD_{Blank}$

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}: $OD_{Sample} - OD_{Blank}$

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

5: Dilution factor of sample in preparation of supernatant

12. Performance Characteristics

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

Analysis

Take 100 µL of human serum sample, 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:

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

Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent touching 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.

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

Notes:

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