（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K245-M

Specification：48T(32 samples)/96T(80 samples)

Measuring instrument：Microplate reader（620-690 nm）

Detection range：0.004-2.0 mmol/L

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

This manual must be read attentively and completely before using this product.

If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623

Tell: 1-832-243-6086

Fax: 1-832-243-6017

Email: techsupport@elabscience.com

Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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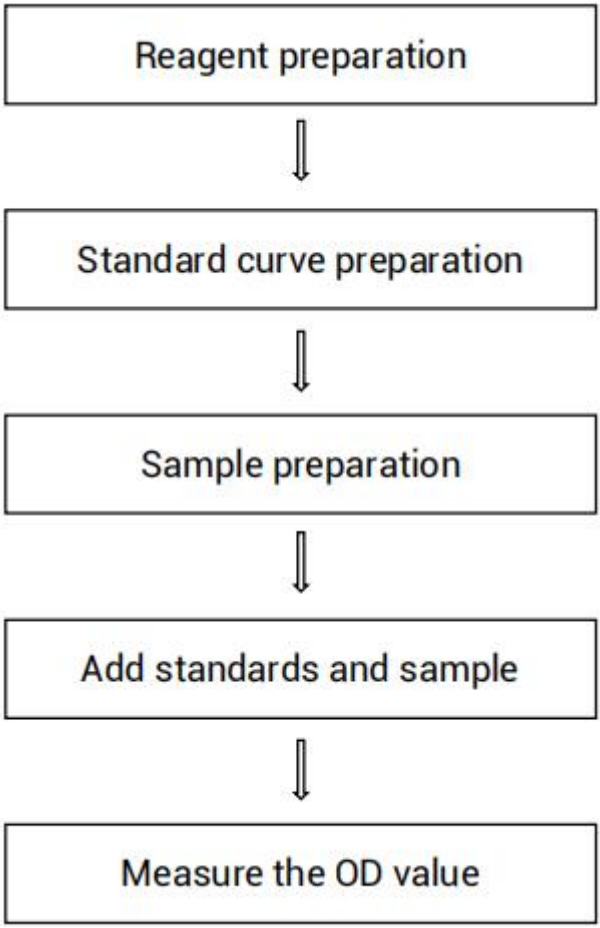
Assay summary



Intended use

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

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	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Chromogenic Agent A	10 mL×1 vial	20 mL×1 vial	2-8°C, 12 months
Reagent 2	Chromogenic Agent B	Powder × 1 vial	Powder × 2 vials	2-8°C, 12 months shading light
Reagent 3	Chromogenic Agent C	Powder × 1 vial	Powder × 2 vials	2-8°C, 12 months shading light
Reagent 4	Protein Precipitator	20 mL × 1 vial	40 mL × 1 vial	2-8°C, 12 months
Reagent 5	10 mmol/L Phosphorus Standard	1 mL × 1 vial	1 mL × 1 vial	2-8°C, 12 months
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces		
	Sample Layout Sheet	1 piece		

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. For a small volume of reagents, please centrifuge

before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

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

Reagents:

Double distilled water, Normal saline (0.9% NaCl)

Reagent preparation

① The preparation of chromogenic agent B working solution：
Dilute one vial of chromogenic agent B with 10 mL of double distilled water, mix well. Store at 2-8°C for 5 days.

② The preparation of chromogenic agent C working solution：
Dilute one vial of chromogenic agent C with 10 mL of double distilled water, mix well. Store at 2-8°C for 2 months.

③ The preparation of chromogenic agent：
For each well, prepare 200 μL of chromogenic agent (mix well 80 μL of double distilled water, 40 μL of chromogenic agent A, 40 μL of chromogenic agent B working solution and 40 μL of chromogenic agent C working solution). The chromogenic agent should be prepared on spot.

④ The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 10 mmol/L phosphorus standard 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. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**0.1**	**0.2**	**0.5**	**0.8**	**1.0**	**1.5**	**2.0**
10 mmol/L standard (mL)	0	0.01	0.02	0.05	0.08	0.1	0.15	0.2
Normal saline (mL)	1	0.99	0.98	0.95	0.92	0.9	0.85	0.8

Sample preparation

① Sample preparation

Serum and plasma: detect directly. If not detected on the same day, the serum or plasma can be stored at -80°C for a month.

Urine: collect fresh urine and centrifuge at 10000×g for 15 min at 4°C. Take the supernatant and preserve it on ice for detection.

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold normal saline (0.9% NaCl).

③ Homogenize 20 mg tissue in 180 μL normal saline (0.9% NaCl) with a dounce homogenizer at 4°C.

④ Centrifuge at 10000×g for 10 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② **Dilution of sample**

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). For the dilution of other sample types, please do pretest to confirm the dilution factor

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.

Operating steps

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

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

③ Mix well 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.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard # ①) from all standard readings. This is the absoluted OD value.

3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

1. Serum (plasma) sample:

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

2. Tissue sample:

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

[Note]

ΔA_{660}: $OD_{Sample} - OD_{Blank}$.

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

5: Dilution factor of sample in preparation of supernatant.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	0.25	0.78	1.40
%CV	2.5	2.3	1.5

Inter-assay Precision

Three human serum samples were assayed 17 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	0.25	0.78	1.40
%CV	3.3	2.6	3.1

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 101%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (mmol/L)	0.15	0.65	1.3
Observed Conc. (mmol/L)	0.2	0.7	1.3
recovery rate(%)	101	103	99

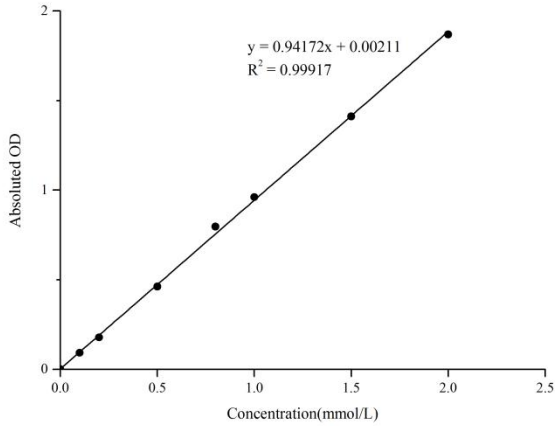
Sensitivity

The analytical sensitivity of the assay is 0.004 mmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only：

Concentration (mmol/L)	0	0.1	0.2	0.5	0.8	1	1.5	2
OD value	0.053	0.144	0.231	0.517	0.870	1.008	1.473	1.910
	0.049	0.142	0.227	0.508	0.824	1.016	1.451	1.929
Average OD	0.051	0.143	0.229	0.512	0.847	1.012	1.462	1.919
Absoluted OD	0.000	0.092	0.178	0.461	0.796	0.961	1.411	1.868



Appendix Π Example Analysis

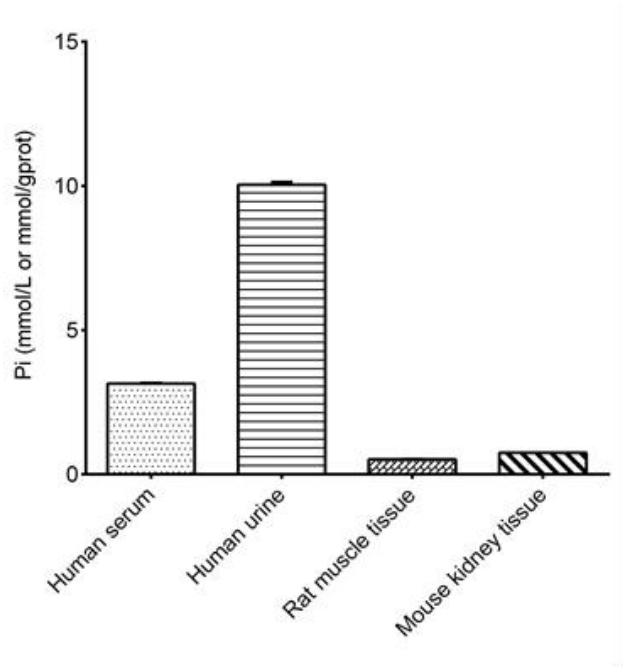
Example analysis：

Take 100 μL of human serum sample carry the assay according to the operation steps. 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:

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

Detect human serum, human urine (dilute for 2 times), 10% rat muscle tissue homogenate (the concentration of protein is 3.98 gprot/L) and 10% mouse kidney tissue homogenate (the concentration of protein is 5.00 gprot/L) according to the protocol, the result is as follows：



Appendix III Publications

1. Wei S , Amevor F K , Du X ,et al.Quercetin mitigates iron-induced cell death in chicken granulosa cell[J].Journal of Animal Science & Biotechnology, 2024, 15(1).DOI:10.1186/s40104-024-01118-0.
2. Yang Y , Tan L , He S ,et al.Sub-MIC vancomycin enhances the antibiotic tolerance of vancomycin-intermediate Staphylococcus aureus through downregulation of protein succinylation[J].Microbiological Research, 2024, 282(000):8.DOI:10.1016/j.micres.2024.127635.
3. Li T .Enhancement of Calcium Chelating Activity in Peptides from Sea Cucumber Ovum through Phosphorylation Modification[J].Foods, 2024, 13.DOI:10.3390/foods13121943.
4. Wang J Y , Hsu H Y , Liu S T ,et al.Ammonia exposure impairs bone mineralization in zebrafish (Danio rerio) larvae[J].Comparative Biochemistry and Physiology, Part C, 2025, 287.DOI:10.1016/j.cbpc.2024.110040.
5. Sun Z , Feng X , Shi Y ,et al.Sodium alginate improves phytase stability and enhances soil phosphorous utilization[J].Biocatalysis and Agricultural Biotechnology, 2024, 61.DOI:10.1016/j.bcab.2024.103372.

Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.