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

Catalog No：E-BC-K245-S

Specification：50 Assays(48 samples)/ 100 Assays(96 samples)

Measuring instrument：Spectrophotometer（660 nm）

Detection range：0.005-2.0 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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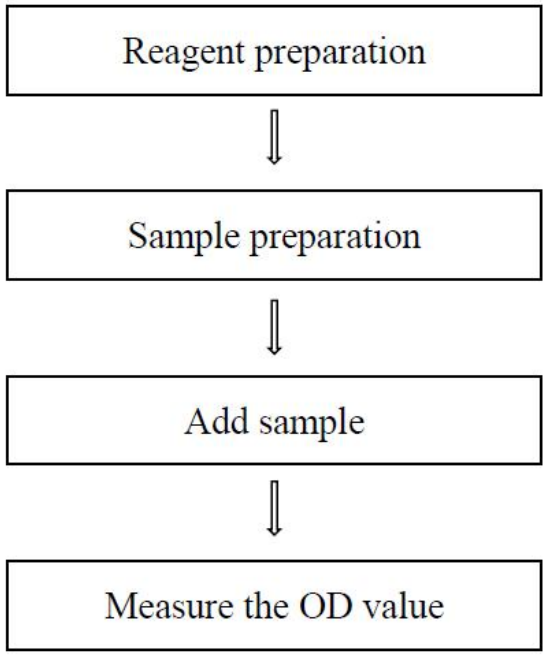
Assay summary



Intended use

This kit can be used to measure the phosphorus (Pi) content in serum, plasma, tissue and other 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 (50 assays)	Size 2 (100 assays)	Storage
Reagent 1	Chromogenic Agent A	25 mL × 1 vial	50 mL × 1 vial	2-8°C, 12 months
Reagent 2	Chromogenic Agent B	Powder × 2 vials	Powder × 4 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	30 mL × 1 vial	60 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

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:

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

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 12.5 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 25 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 2 mL of chromogenic agent (mix well 0.8 mL of double distilled water, 0.4 mL of chromogenic agent A, 0.4 mL of chromogenic agent B working solution and 0.4 mL of chromogenic agent C working solution). The chromogenic agent should be prepared on spot.

④ The preparation of 0.5 mmol/L standard solution：
For each well, prepare 200 μL of 0.5 mmol/L standard solution (mix well 10 μL of 10 mmol/L phosphorus standard and 190 μL of double distilled water). The 0.5 mmol/L standard solution should be prepared on spot.

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.

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 30 mg tissue in 270 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
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. For the dilution of other sample types, please do pretest to confirm the dilution factor

Operating steps

The preparation of sample supernatant

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

The measurement of samples

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 the tube.
Sample tube: Take 0.2 mL of sample supernatant to the corresponding tube.

② Add 2.0 mL of chromogenic agent to each tube and mix well.

③ Incubate the tubes at 37°C 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.

Calculation

The sample:

1. Serum (plasma) sample:

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

2. Tissue sample:

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

[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 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)	0.26	1.05	1.65
%CV	1.3	0.9	0.8

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)	0.26	1.05	1.65
%CV	1.2	1.4	1.3

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 102%.

	Sample 1	Sample 2	Sample 3
Expected Conc. （mmol/L）	0.55	1.25	1.75
Observed Conc. （mmol/L）	0.5	1.3	1.8
recovery rate(%)	99	106	101

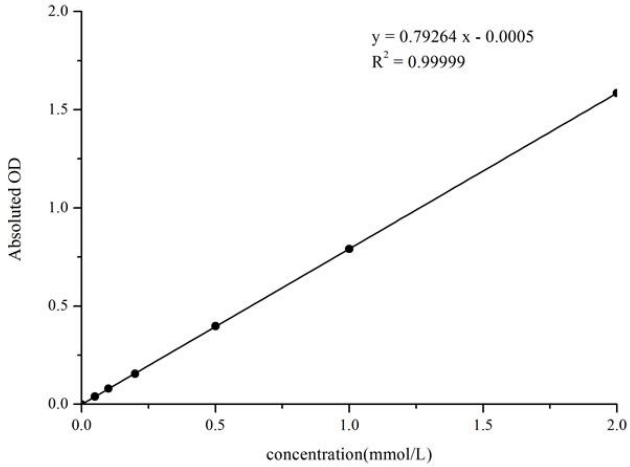
Sensitivity

The analytical sensitivity of the assay is 0.005 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**	**0.05**	**0.1**	**0.2**	**0.5**	**1**	**2**
Average OD	0.026	0.065	0.106	0.181	0.424	0.817	1.611
Absoluted OD	0	0.039	0.08	0.155	0.398	0.791	1.585



Appendix II Example Analysis

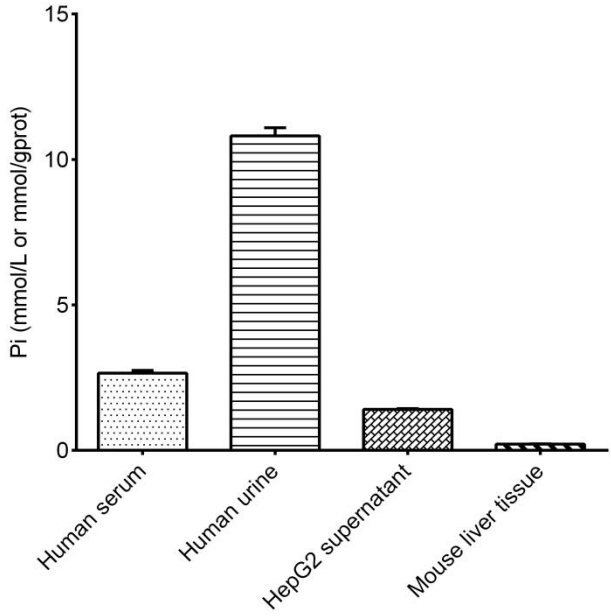
Example analysis:

Take 0.1 mL of human serum, carry the assay according to the operation steps. 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 well is 0.373, and the calculation result is:

$$\underset{(\text{mmol/L})}{\text{Pi}} = \frac{0.396 - 0.011}{0.373 - 0.011} \times 0.5 \times 5 \times 1 = 2.66\ (\text{mmol/L})$$

Detect human serum, human urine (dilute for 2 times), 10% mouse liver tissue homogenate (the concentration of protein is 11.99 gprot/L), culture supernatant of HepG2 cells according to the protocol, the result is as follows:



Appendix III Publications

1. 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.
2. Sun Z, Yue Z, Liu H, et al. Microbial-Assisted Wheat Iron Biofortification Using Endophytic Bacillus altitudinis WR10[J]. Frontiers in Nutrition, 2021:476-. DOI: 10.3389/fnut.2021.704030.
3. 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: Toxicology & Pharmacology, 2025, 287(000). DOI:10.1016/j.cbpc.2024.110040.
4. 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(000). DOI: 10.1016/j.bcab.2024.103372.
5. Abo-Elenin M H H, Kamel R, Nofal S, et al. The crucial role of beta-catenin in the osteoprotective effect of semaglutide in an ovariectomized rat model of osteoporosis[J]. Naunyn-Schmiedeberg's Archives of Pharmacology, 2025, 398(3):2677-2693. DOI: 10.1007/s00210-024-03378-z.

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.