TECHNICAL MANUAL

Phosphorus (Pi) Colorimetric Assay Kit (Phospho Molybdate)

- **SKU CODE:** MAES0417
- **SIZE:** 100 Assays
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Sample preparation
- Preparation of sample supernatant
- Add chromogenic agent
- Measure optical density at 660 nm

2. Intended use

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

3. Detection principle

Inorganic phosphorus reacts with molybdic acid to produce phosphomolybdic acid. Phosphomolybdic acid can be reduced to molybdenum blue under the action of reducing agent. The molybdenum blue has a maximum absorption peak at 660 nm. The phosphorus content can be calculated indirectly by measuring the optical density value at 660 nm.

4. 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, protected from light
Reagent 3	Chromogenic Agent C	Powder × 1 vial	Powder × 2 vials	2-8°C, 12 months, protected from 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

5. Materials prepared by users

Instruments:

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

Reagents:

Double distilled water, Normal saline (0.9% NaCl)

6. Reagent preparation

1. **Preparation of chromogenic agent B working solution:** Dilute one vial of chromogenic agent B with 12.5 mL of double distilled water and mix well. Store at 2-8°C for 5 days.
2. **Preparation of chromogenic agent C working solution:** Dilute one vial of chromogenic agent C with 25 mL of double distilled water and mix well. Store at 2-8°C for 2 months.
3. **Preparation of chromogenic agent:** For each well, prepare 2 mL of chromogenic agent by mixing 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 immediately before use.
4. **Preparation of 0.5 mmol/L standard solution:** For each well, prepare 200 µL of 0.5 mmol/L standard solution by mixing 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 immediately before use.

7. 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 10,000×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 (MAES0177).

2. **Dilution of sample:** The recommended dilution factor for different samples is as follows (for reference only): Human serum: 1 Human plasma: 1 10% Mouse kidney tissue homogenate: 1 10% Mouse heart tissue homogenate: 1 10% Mouse liver tissue homogenate: 1 Note: The diluent is double distilled water. For the dilution of other sample types, please perform a pretest to confirm the dilution factor.

8. 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 and mix fully. Centrifuge at 1,100×g for 10 minutes 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 minutes, then cool the tubes to room temperature.

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

9. Calculation

The sample:

1. Serum (plasma) sample:

$Pi\ (mmol/L) = (\Delta A_1/\Delta A_2) \times c \times 5 \times f$

2. Tissue sample:

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

[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)

10. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

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

Intra-assay Precision

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.

Inter-assay Precision

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%.

Recovery

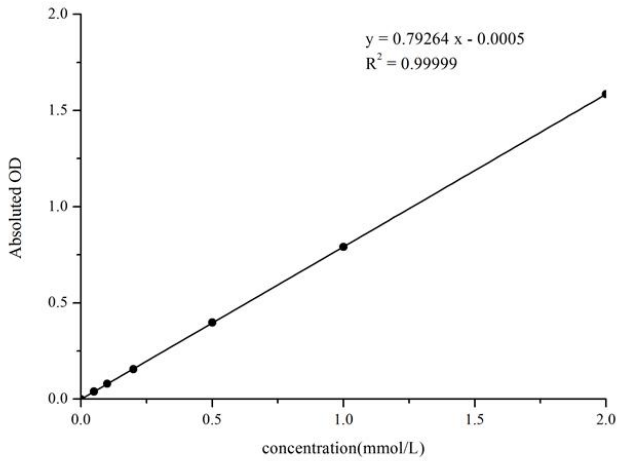
Sample	Expected Conc. (mmol/L)	Observed Conc. (mmol/L)	Recovery rate (%)
Sample 1	0.55	0.5	99
Sample 2	1.25	1.3	106
Sample 3	1.75	1.8	101

Sensitivity

The analytical sensitivity of the assay is 0.005 mmol/L. This was determined by adding two standard deviations to the mean optical density obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Standard curve

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



Standard curve

Concentration (mmol/L)	Average OD	Absolute OD
0	0.026	0
0.05	0.065	0.039
0.1	0.106	0.08

Concentration (mmol/L)	Average OD	Absolute OD
0.2	0.181	0.155
0.5	0.424	0.398
1	0.817	0.791
2	1.611	1.585

11. Appendix II Example Analysis

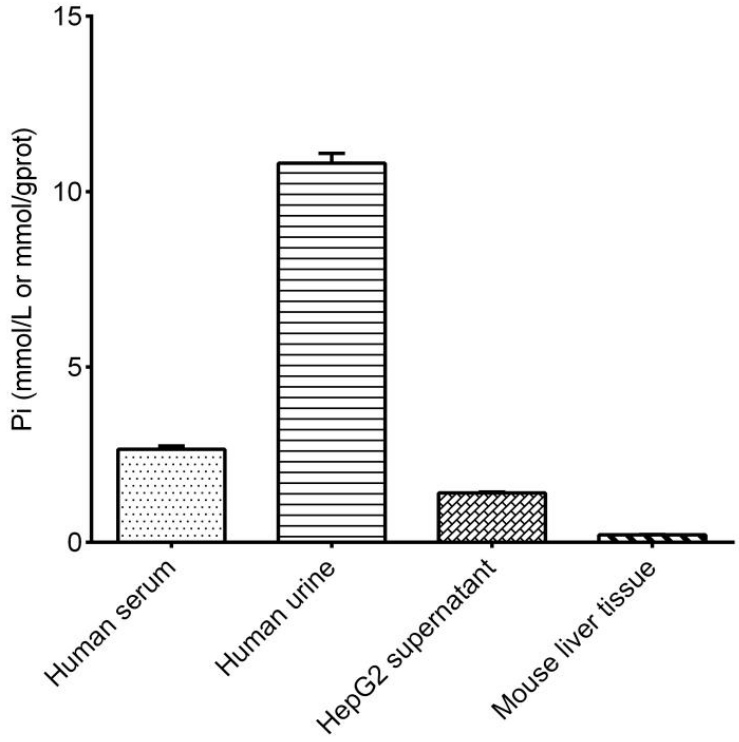
Example analysis:

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

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

Pi (mmol/L) = (0.396 - 0.011)/(0.373 - 0.011) × 0.5 × 5 × 1 = 2.66 (mmol/L)

Detect human serum, human urine (diluted 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:



12. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.

2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.

3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.

4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.

5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.

6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

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Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.