



TECHNICAL MANUAL

Phosphorus (Pi) Colorimetric Assay Kit (Phospho Molybdate)

- **SKU CODE:** MAES0161
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

1. Assay summary

- Preparation of sample supernatant
- Standard well preparation
- Sample well preparation
- Add chromogenic agent
- Incubate and measure OD value

2. Intended use

This kit can be used to measure the phosphorus (Pi) content in serum, plasma, urine and tissue 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 OD value at 660 nm.

4. Kit components & storage

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

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
	Plate Sealer	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

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

Reagents:

Double distilled water, Normal saline (0.9% NaCl)

6. 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 fresh.
- 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 serial concentrations. The recommended dilution gradient is as follows: 0, 0.1, 0.2, 0.5, 0.8, 1, 1.5, 2 mmol/L.

7. Sample preparation

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 10,000 x 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 μ L normal saline (0.9% NaCl) with a dounce homogenizer at 4 °C.
4. Centrifuge at 10,000 x 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).

Dilution of sample:

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

- 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 a pretest to confirm the dilution factor.

8. The key points of the assay

1. Prevent the formation 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.

9. 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 1,100 x 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 concentrations 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.

10. 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 absolute OD value.

3. Plot the standard curve by using absolute OD value of standard and corresponding 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:

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

2. Tissue sample:

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

[Note]

ΔA_{660} : $OD_{\text{Sample}} - OD_{\text{Blank}}$.

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

5: Dilution factor of sample in preparation of supernatant.

11. 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)

Inter-assay Precision

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

Recovery

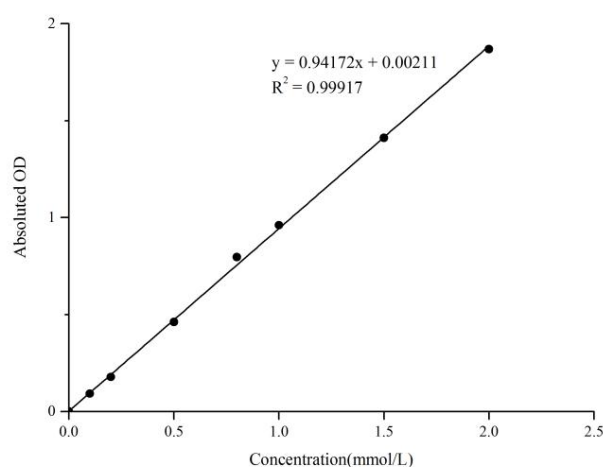
Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times in parallel to get the average recovery rate of 101%.

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.

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:



12. Appendix II Example Analysis

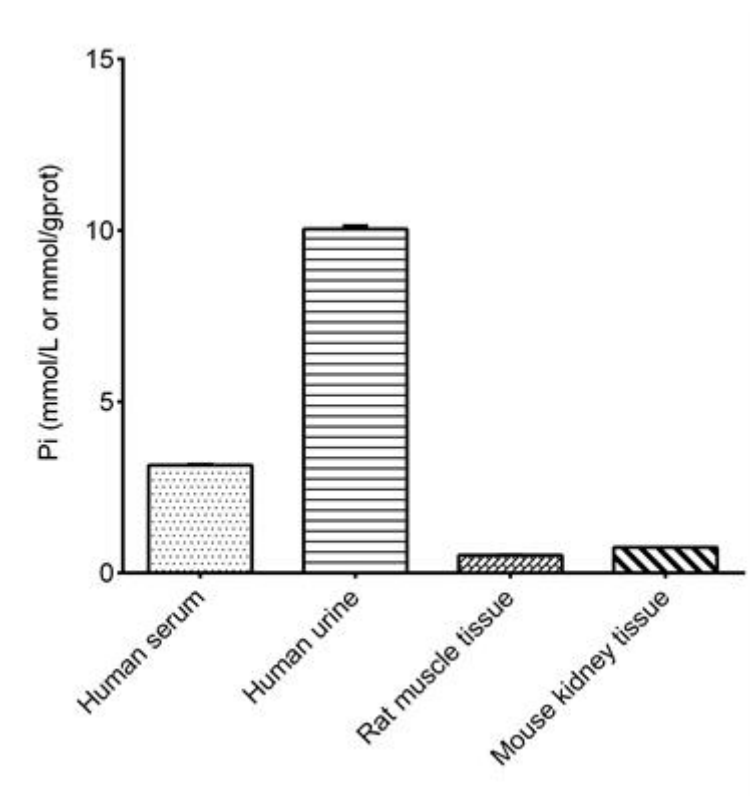
Example analysis:

Take 100 μ L of human serum sample and carry out the assay according to the operation steps. The results are as follows:

Standard curve: $y = 0.9268x + 0.0059$, 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:

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

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:



13. Statement

1. This assay kit is for Research Use Only. Assay Genie will not be responsible for any arising problems or legal responsibilities caused by using the kit for clinical diagnosis or other purposes.
2. Please read the instructions carefully and calibrate 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. Assay Genie 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.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.



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