Phosphate Assay Kit

(Cat/No.:BC061 Size:100T / 96S)

1. Principle（Phosphomolybdic acid method）

Inorganic phosphate in the sample reacts with molybdate to form phosphomolybdate, which is then reduced to molybdenum blue, showing a maximum absorption peak at 660 nm. The inorganic phosphate content can be calculated by colorimetry.

2. Reagents and Preparation (The kit is valid for 6 months)

Reagent 1: 50 mL ×1 bottle, store at 4℃.

Reagent 2: Powder ×2 bottles, store at 4℃; before use, dissolve each bottle in 40 mL deionized water. Prepared solution can be stored at 4℃ protected from light for 1–2 weeks.

Reagent 3: Powder ×1 bottle, store at 4℃; before use, dissolve in 50 mL deionized water. Prepared solution can be stored at 4℃ for 2 months.

Working solution preparation: Mix Reagent 1: Reagent 2: Deionized water: Reagent 3 in a ratio of 1:1:2:1. The prepared working solution should be light yellow (if it turns green or black, the chromogenic reagent is invalid). Store at 4℃ protected from light and use within 2 days.

Reagent 4: 10 mmol/L phosphate standard solution, **1 mL ×1**, store at 4℃. **Before use, dilute a portion** 20-fold with deionized water to prepare **0.5 mmol/L phosphate working standard solution**.

Precipitating reagent: 50 mL ×1 bottle, store at 4℃.

3. Required Instruments and Reagents

Visible spectrophotometer and 1 cm path length cuvette (or microplate reader (660 nm) and 96-well plate), vortex mixer, 37℃ water bath, centrifuge, deionized water.

4. Procedure

1. Sample Preparation:

① **Serum (plasma) or other liquid** samples: use directly.

② **Animal tissue:**： weigh (~0.05–0.1 g), add deionized water at a ratio of tissue weight (g) : volume (mL) = 1:10 (or 1:5 for low content), homogenize on ice, centrifuge at 4000 rpm for 10 min, collect the supernatant for assay (protein concentration can be measured).

③ **Bacteria/cell samples:** collect bacteria or cells in a centrifuge tube (remove culture medium), add 0.5 mL deionized water per 5×10^6 cells, sonicate on ice (power 20% or 200 W, 5 s on, 15 s off, repeat 5–10 times), centrifuge at 4000 rpm for 10 min, collect supernatant for assay (protein concentration can be measured).

④ **Plant tissue samples: Method 1:** Wash plant tissue with deionized water, blot dry, cut and grind into powder in a mortar with liquid nitrogen, weigh powder, add 9-fold

volume of deionized water (weight (g) : volume (mL) = 1:9), vortex (or use homogenizer) 1 min, centrifuge at 4000 rpm for 10 min, collect supernatant. **Method 2:** Wash and blot dry, weigh, add 4-fold volume of deionized water (weight (g) : volume (mL) = 1:4), mechanically homogenize on ice, centrifuge at 4000 rpm for 10 min, collect supernatant. **(Note: High-water-content plants are generally treated with Method 2 and sample-to-reagent ratio can be adjusted; low-water-content or dry samples are recommended for Method 1.)**

2. Sample Pretreatment:

Add 0.1 mL of prepared sample or supernatant to 0.4 mL precipitating reagent (**for plant homogenates, mix 0.25 mL sample + 0.25 mL precipitating reagent**), mix thoroughly, centrifuge at 4000 rpm for 10 min, and use the supernatant for detection according to the table below.

3. Operation Table:

	Test Tube	Standard Tube	Blank Tube
Supernatant (mL)	0.2		
0.5 mmol/L phosphate working solution (mL)		0.2	
Deionized water (mL)			0.2
Working solution (mL)	2	2	2
Mix, incubate in 37℃ water bath for 30 min, cool to room temperature, measure absorbance at 660 nm, path length 1 cm, zero with deionized water. (Alternatively, add 200 μL of each solution to a 96-well plate, read at 660 nm on a microplate reader, calculation remains the same.)			

5. Calculation Formula

1. Calculation for liquid samples (serum, plasma, etc.):

$$\text{Phosphorus content (mmol/L)} = \frac{A_{sample} - A_{blank}}{A_{standard} - A_{blank}} \times C_{standard} \times N$$

2. Calculation for animal tissue or cells based on protein concentration:

$$\text{Inorganic phosphorus content (mmol/g Protein)} = \frac{A_{sample} - A_{blank}}{A_{standard} - A_{blank}} \times C_{standard} \times N \div Cpr$$

3. Calculation for animal or plant tissue based on fresh weight:

$$\text{Inorganic phosphorus content (mmol/g Fresh weight)} = \frac{A_{sample} - A_{blank}}{A_{standard} - A_{blank}} \times C_{standard} \times N \div \frac{W}{V_{sample\ total}}$$

4. Calculation for cell samples based on cell number:

$$\text{Inorganic phosphorus content (mmol/}10^6 Cell) = \frac{A_{sample} - A_{blank}}{A_{standard} - A_{blank}} \times C_{standard} \times N \div \frac{\text{Total cell number}}{V_{sample\ total}}$$

In the formulas above:

$C_{standard}$: Standard concentration, 0.5 mmol/L

N: Dilution factor during sample pretreatment, 5 or 2 (plants)

Cpr: Protein concentration of homogenate, g/L

W: Fresh weight of sample, g
$V_{sample\ total}$: Total volume of deionized water added during homogenization, L
Total cell number: Number of cells before homogenization, 10^6 cells

I. Example:

Take 0.1 mL rat serum, add 0.4 mL precipitating reagent, then measure according to the operation table. If OD values are: Blank tube 0.012, Standard tube 0.421, Test tube 0.395, the calculation is:

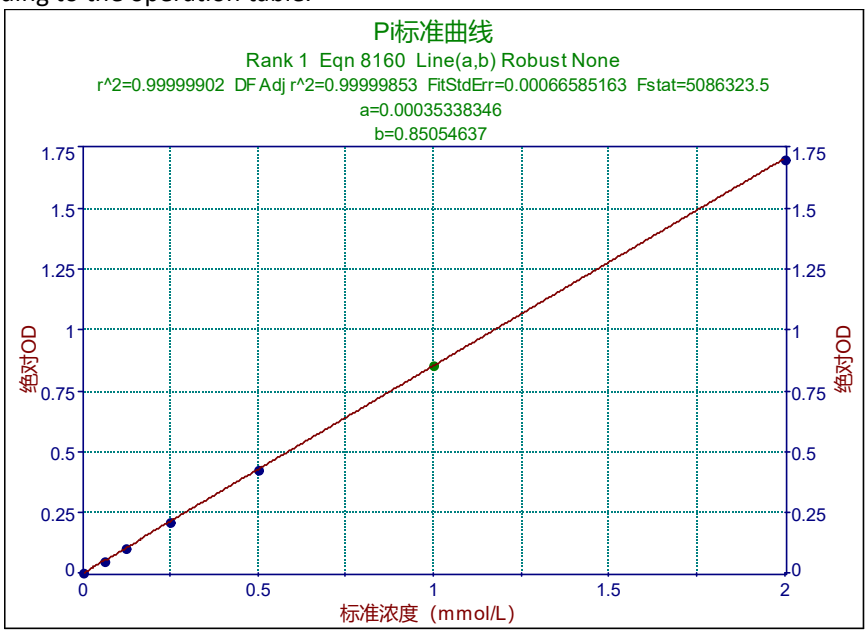
$$\text{Rat serum phosphorus content (mmol/L)} = \frac{0.395 - 0.012}{0.421 - 0.012} \times 0.5 \times 5$$
$$= 2.34\text{mmol/L}$$

II. Related Technical Parameters:

	Parameter	Value
1	Detection limit	0.02 mmol/L
2	Linear range	0.02–2 mmol/L
3	Intra-batch CV	2.3%
4	Inter-batch CV	3.1%
5	Color stability	1 h
6	Recovery	101%

III. Standard Curve Preparation:

Dilute 10 mmol/L phosphate standard stock solution with deionized water to different concentrations (0, 0.0625, 0.125, 0.25, 0.5, 1, 2 mmol/L), and prepare standard curve according to the operation table.



(Standard curve is optional; the assay can be performed directly according to the operation table and calculation formulas without affecting results.)