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Phosphorus (Pi) Assay Kit
(Phospho Molybdate Method)

Catalog No.: BC00072

Size: 100T

If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉Email (Sale) order@enkilife.com

✉Email (Techsupport) techsupport@enkilife.com

Tel: 0086-27-87002838

Website: www.enkilife.com

Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

Product Name	Phosphorus (Pi) Assay Kit (Phospho Molybdate Method)
Detection Methods	Colorimetric
Sample type	Serum, plasma, tissue
Detection Type	Quantitative
Detection instrument and wavelength	Microplate reader (620-690 nm, optimal detection wavelength is 660 nm)
Range	0.25-2mM
Sensitivity	0.0232mM

Product Introduction

Phosphorus exists in organisms in two forms: inorganic phosphorus (phosphate) and organic phosphorus, and is involved in processes such as energy, nucleic acid metabolism, and protein phosphorylation. Determining the total phosphorus and inorganic phosphorus content in crops helps evaluate the efficiency of phosphorus utilization and provides a scientific basis for precision fertilization.

Features

★ Wide applicability: Suitable for a variety of sample types, including serum, plasma, tissue samples , etc.

Detection Principle

Inorganic phosphorus reacts with molybdic acid to form phosphomolybdic acid. Under the action of a reducing agent, phosphomolybdic acid is reduced to molybdenum blue, which has a maximum absorption peak at 660 nm. Within a certain range, the absorbance value is proportional to the concentration. When this kit detects tissue samples, the total protein concentration needs to be determined. It is recommended to use the protein concentration determination kit (BCA method) (Cat. No.: BC00006).

Product Composition

Serial Number	Product Name	Packing Specifications	Storage
Reagent 1	Chromogen A	20 mL	Store at -20℃ , store at 2-8 ℃ after
Reagent 2	Chromogen B	powder	Store at -20℃ away from light , store

Reagent 3	Chromogen C	powder	Store at -20℃ away from light , store
Reagent 4	Protein Precipitants	40 mL	Store at -20℃ , store at 2-8 ℃ after
Reagent 5	2 mmol/L Phosphorus	1 mL	Store at -20℃ , store at 2-8 ℃ after
	96-well ELISA plate	1 plate	RT
	96-well membrane	2 pieces	RT

Note: The reagents must be stored strictly according to the storage conditions in the table above. Reagents from different test kits cannot be mixed. For reagents with a small volume, centrifuge them before use to avoid insufficient amount of reagent.

Storage conditions

The unopened kit can be stored at -20°C for 6 months.

Preparation before the experiment

Sample processing

1. Liquid samples such as serum and plasma: directly measure.
2. Tissue sample: Take 0.020-1.0 g of fresh tissue block, rinse with 2-8℃ normal saline (0.9% NaCl) to remove blood, absorb with filter paper, weigh, put into a homogenization container, add normal saline (0.9% NaCl) at a ratio of weight (g): volume (mL) = 1:9, homogenize, centrifuge at 4℃, 10000×g for 10 min, take the supernatant and place on ice for testing.

· Preparation of the kit

1. Before testing, the reagents in the kit were equilibrated to room temperature.
2. Preparation of reagent 2 working solution: Take a bottle of reagent 2, add 10 mL of double distilled water to it, dissolve it fully, and store it at 2-8℃ away from light for 5 days.
3. Preparation of reagent 3 working solution: Take a bottle of reagent 3, add 10 mL of double distilled water to it, dissolve it fully, and store it at 2-8℃ for 2 months.
4. Color developer working solution: Mix double distilled water: reagent 1: reagent 2 working solution: reagent 3 working solution in a volume ratio of 2:1:1:1, prepare before use, and store at 2-8℃ away from light for 24 h.
5. Dilution of standards of different concentrations: The initial concentration of the standard was 2 mmol/L, which was diluted in half with double distilled water. The concentration gradient was: 2, 1, 0.5, 0.25, 0.125, 0 (blank well) mmol/L. It was divided into 6 groups.

Key points of the experiment

Be careful to prevent phosphorus pollution when using, and prepare the color developer before

use.

Operation process

1. Preparation of supernatant: Take 0.1mL serum (plasma) or 10% tissue homogenate in a 1.5mL EP tube, add 0.4mL reagent IV, mix thoroughly, centrifuge at 1100×g for 10 min, and take the supernatant for testing.
2. Standard wells: Take 35 μL of standard solutions of different concentrations and add them to the corresponding standard wells.
3. Sample wells: Take 35 μL of sample supernatant and add it to the corresponding sample wells.
4. Add 200 μL of the colorimetric working solution to the standard wells and sample wells in step (1).
5. The plate was shaken on a microplate reader for 10 s, incubated at 37°C for 30 min, and the OD value of each well was measured at 660 nm.

Operation table

	Standard wells	Assay well
Standards of different	35	--
Supernatant of sample to be	--	35
Chromogenic working solution	200	200
The plate was shaken on a microplate reader for 10 s, incubated at 37°C for 30 min, and the OD value of each well was measured at 660 nm.		

Result calculation

Standard fitting curve: y = ax + b

The formula for calculating phosphorus concentration in serum (plasma) is:

Pi (mmol/L) = (ΔA660 - b) ÷ a × 5 × f

The formula for calculating phosphorus concentration in tissues is:

Pi (mmol/gprot)= (ΔA660 - b) ÷ a × 5 × f ÷ Cpr

annotation:

y: OD value of standard well - OD value of blank well (OD value when the concentration of standard is 0)

x: concentration of the standard

a: Slope of the standard curve

b: standard curve intercept

ΔA660: Sample OD value - blank OD value (OD value when the standard concentration is 0)

f: dilution factor of the sample before adding it to the detection system

Cpr: sample protein concentration (gprot/L)

5: The number of times the sample was diluted during the supernatant preparation process

Notes

1. The phosphorus determination reagent (1ml 5mol/L H_2SO_4+0.13556g ammonium molybdate+9ml water+0.0941g ferrous sulfate heptahydrate) needs to be prepared and used immediately. The preparation order is water first and then acid, and it cannot be reversed.
2. The kit is for research use only. If it is used for clinical diagnosis or any other purpose, our company will not be responsible for any problems arising therefrom and will not bear any legal liability.
3. Please read the instructions carefully and adjust the instrument before the experiment, and conduct the experiment strictly in accordance with the instructions.
4. Please wear lab coats and latex gloves for protection during the experiment.
5. The detection range of the kit is not equivalent to the concentration range of the analyte in the sample. If the concentration of the analyte in the sample is too high or too low, please dilute or concentrate the sample appropriately.
6. If the sample being tested is not among the sample types listed in the instructions, it is recommended to conduct a preliminary experiment to verify the effectiveness of the test.
7. The final experimental results are closely related to the effectiveness of the reagents, the relevant operations of the experimenter, the experimental environment and other factors. Our company is only responsible for the kit itself, not for the sample consumption caused by the use of the kit. Please fully consider the possible usage of the sample before use and reserve sufficient samples.