Tissue Total Phosphorus Content Assay Kit

Note: The reagents have been changed, so please be aware of and follow this instruction strictly.

Detection instrument: Spectrophotometer /Microplate reader

Catalog Number: BC2855

Size: 100T/96S

Components:

Reagent I: Liquid 22 mL×1, store at 2-8℃. It is strong corrosive and strong oxidizing. Tighten the cover immediately after using.

Reagent II: Liquid 7 mL×1, store at 2-8℃.

Reagent IIIA: Powder×1, store at 2-8℃. Add 5 mL of distilled water to fully dissolve. Unused reagent is still stored at 2-8℃ for four weeks.

Reagent IIIB: Powder×1, store at 2-8℃. Add 5 mL of distilled water to fully dissolve. Unused reagent is still stored at 2-8℃ for four weeks.

Reagent III: Reagent IIIA, Reagent IIIB and Reagent II are mixed by the ratio of 1:1:1 to make Reagent III before use. Prepared Reagent III is light yellow. It is colorless if the reagent is invalid. It is blue if the reagent is contaminated with phosphorus. Prepared Reagent III could only be used the same day.

Standard: Liquid 1 mL×1, 10 mmol/L inorganic phosphorus standard, store at 2-8℃.

Product Description:

The form of phosphorus includes inorganic phosphorus and organic phosphorus. Inorganic phosphorus mainly refers to phosphate radical, which is involved in many kinds of metabolism, including energy metabolism, nucleic acid metabolism, protein phosphorylation, dephosphorylation, and so on. By measuring the content of total phosphorus and inorganic phosphorus, the utilization rate of phosphorus in crops can be understood, and the basis for rational fertilization can be provided.

After digestion, total phosphorus was converted into inorganic phosphorus. The molybdenum blue method is a classical method for determining the content of inorganic phosphorus. Under certain conditions, molybdenum blue and phosphate form a substance with a characteristic absorption peak at 660nm. By measuring the light absorption of 660nm, the inorganic phosphorus content can be calculated, and then the total phosphorus content in the tissue can be calculated.

Required Material

Centrifuge, spectrophotometer/microplate reader, water bath, micro glass cuvette/96 well flat-bottom plate, transferpettor, distilled water, **concentrated sulfuric acid** (99%).

Procedure:

I. Sample Extraction:

0.1g of sample with 1mL of concentrated sulfuric acid (Wrap the sealing film to prevent the lid from bursting) put into boiling water bath for 10 minutes. When the solution is black or brown, take it out. Add

200μL of Reagent I after cooling, mix well. Continue boiling (Wrap the sealing film to prevent the lid from bursting) until the solution is transparent, then cool at room temperature, add 3.8mL of distilled water and

mix well. centrifugated at 10000rpm and room temperature for 10 minutes, supernatant is used for test.

II. Determination procedure:

1. Preheat spectrophotometer/microplate reader for more than 30min, adjust the wavelength to 660nm and set spectrophotometer counter to zero with distilled water.
2. Set the temperature of water bath to 40℃.
3. Preparation of 1 mmol/L standard solution: 100 μL 10 mmol/L phosphorus standard solution and 900 μL distilled water are mixed to prepare 1 mmol/L standard solution.
4. Add reagents with the following list:

Reagent (μL)	Blank tube (A_B)	Standard tube (A_T)	Test tube (A_S)
Standard	-	10	-
Supernatant	-	-	10
Distilled water	100	90	90
Reagent III	100	100	100

Mix well, 40℃ water bath for 10 minutes, detect the absorbance at 660 nm after cooling at room temperature for 10 minutes. Record as A_B, A_S and A_T respectively. Standard tube and blank tube only need to be measured once or twice.

III. Calculation:

Total phosphorus content (mmol/g weight)=$[C\times(A_T- A_B)\div(A_S - A_B)]\times V\div W$

$=0.005\times(A_T - A_B)\div(A_S - A_B)\div W$

C: standard concentration, 1mmol/L;

V: supernatant volume, 5 mL=0.005 L;

W: Sample weight, g.

Note:

1. When the determination of A is greater than 1.5, it is recommended to dilute supernatant with distilled water before performing the measurement and multiply the dilution factor in the calculation formula.

Experimental example:

1. Take 0.1g kidney according to the extraction procedure, centrifugally take it up and clean it, and then follow the measurement procedure. Use 96 well plate to calculate: A_T= 0.191, A_B= 0.051, A_S= 0.282. Calculate the total phosphorus content according to the sample mass:
   Total phosphorus content (mmol/g weight) = $0.005 \times (A-A_B) \div (A_S-A_B) \div W$=0.030 mmol/g weight.
2. Take 0.1g spleen according to the extraction procedure, centrifugally take the cleaning, and then follow the measurement procedure. Use 96 well plate to calculate: A_T= 0.197, A_B= 0.051, A_S= 0.282, calculate the total phosphorus content according to the sample mass:

Total phosphorus content (mmol/g weight) =0.005×(A-A_B) ÷(A_S-A_B) ÷W=0.032 mmol/g weight.

Related Products:

BC2860/BC2865	Serum Total Iron Binding Capacity(TIBC) Assay Kit
BC2810/BC2815	Blood Zinc Content Assay Kit
BC2820/BC2825	Water Mercury Ion(Hg^{2+}) Content Assay Kit
BC2840/BC2845	Phosphate Content Assay Kit

Technical Specifications:

The detection limit: 0.0338 mmol/L

Linear range: 0.625-8 mmol/L