

# **Tissue Inorganic Phosphorus Content Assay Kit**

**Note:** It is necessary to predict 2-3 large difference samples before the formal determination.

**Detection instrument:** Spectrophotometer/Microplate reader

Catalog Number: BC2845

Size: 100T/96S

## **Components:**

**Reagent I:** Liquid 110 mL×1, store at 2-8°C. **Reagent II:** Liquid 5 mL×1, store at 2-8°C.

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

**Reagent IIIB:** Powder×1, store at 2-8°C. Add 5 mL of distilled water to fully dissolve. Unused reagent is still stored at 2-8°C 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°C.

## **Product Description:**

Inorganic phosphorus mainly refers to phosphate radical, which is involved in many kinds of metabolism, including energy metabolism, nucleic acid metabolism, protein phosphorylation and dephosphorylation, and so on. In addition, inorganic phosphorus promotes the synthesis, transformation and transport of carbohydrates.

Molybdenum blue can react with phosphate group, the reaction product can be detected by colorimetric assay at 660 nm and calculate the inorganic phosphorus content indirectly.

## Required material

Spectrophotometer/microplate reader, centrifuge, water bath, transferpettor, micro glass cuvette/96 well flat-bottom plate, mortar/homogenizer and distilled water.

# **Procedure:**

## I. Sample Extraction:

Suggested 0.1g of sample with 1mL of Reagent I, fully grinding on ice, centrifuge at 10000 rpm and 4°C for 10 minutes, supernatant is used for test.

## II. Determination procedure:

- 1. Preheat spectrophotometer/microplate reader for 30 minutes, adjust wavelength to 660 nm, set spectrophotometer counter to zero with distilled water.
- 2. Set the temperature of water bath to 40°C.
- 3. Preparation of 1 mmol/L standard solution : 100μL 10 mmol/L phosphorus standard solution and



900µL distilled water mixed to prepare 1 mmol/L standard solution.

4. Add reagents with the following list:

Reagent (µL)	Blank tube (A <sub>B</sub> )	Standard tube (A <sub>T</sub> )	Test tube (A <sub>S</sub> )
Standard	-	10	0/3/
Supernatant	- @	-	10
Distilled water	100	90	90
Reagent III	100	100	100

Mix well, 40°C water bath for 10 minutes, detect the absorbance at 660 nm after cooling at room temperature for 10 minutes. Record as A<sub>B</sub>, A<sub>S</sub> and A<sub>T</sub> respectively. Standard tube and blank tube only need to be measured once or twice. **The results should be determined within 40 minutes.** 

#### III. Calculation:

Inorganic phosphorus content (mmol/g weight) =[ $C \times (A_T - A_B) \div (A_S - A_B)$ ] $\times V \div W$ =0.001 $\times (A_T - A_B) \div (A_S - A_B) \div W$ 

C: standard concentration, 1mmol/L;

V: supernatant volume, 1ml=0.001 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, add 1 mL of Reagent I, centrifugate the supernatant, and then operate according to the determination steps. Use 96 well plate to measure:  $A_T = 0.696$ ,  $A_B = 0.051$ ,  $A_S = 0.282$ .

Inorganic phosphorus content (mmol/g weight) = $0.001 \times (A_T - A_B) \div (A_S - A_B) \div W = 0.028$  mmol/g weight.

2. 0.1g liver is added with 1 mL of Reagent I, and the supernatant is centrifuged. Then, the determination procedure is followed. The 96 well plate is used to determine.  $A_T = 0.578$ ,  $A_B = 0.051$ ,  $A_S = 0.282$ .

Inorganic phosphorus content (mmol/g weight) = $0.001 \times (A_T - A_B) \div (A_S - A_B) \div W = 0.023$  mmol/g weight.

## **Related Products:**

Bu J, Yu J, Wu Y, et al. Hyperlipidemia affects tight junctions and pump function in the corneal endothelium[J]. The American Journal of Pathology, 2020.

#### **Related Products:**

BC2860/BC2865 Serum Total Iron Binding Capacity (TIBC) Assay Kit

BC2850/BC2855 Total Phosphorus Content Assay Kit

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BC4350/BC4355 Tissue Iron Content Assay Kit BC4380/BC4385 Blood Ammonia Content Assay Kit

# **Technical Specifications:**

The detection limit: 0.0302 mmol/L Linear range: 0.625-8 mmol/L