Blood Phosphate Content Assay Kit

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

Operation Equipment: Spectrophotometer

Catalog Number: BC1650

Size: 50T/48S

Components:

Reagent I: Liquid 70 mL×1, store at 2-8℃.

Reagent II: Powder×1, store at 2-8℃. Add 10mL of distilled water mix well. Unused reagent is still stored at 2-8℃ for 4 weeks.

Reagent III: Powder×1, store at 2-8℃. Add 10 mL of distilled water to fully dissolve. Unused reagent is still stored at 2-8℃ for 4 weeks.

Reagent IV: Liquid 10 mL×1, store at 2-8℃

Preparation of working solution: before use according to the sample volume, the reagent II: reagent III: reagent IV = 0.5mL: 0.5mL: 0.5mL (1.5mL, about 3T). The working solution should be light yellow. If it is colorless, the reagent is invalid, if it is blue, it is phosphorus contamination, and it is limited to be used on the same day. (Note: It is best to use new beakers, glass rods, and glass pipettes, or disposable plastic containers to avoid phosphorus contamination.)

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

Description:

Blood phosphorus mainly refers to the inorganic phosphorus in blood, which exists in the form of inorganic phosphorus salt. The concentration of calcium and phosphorus in plasma is closely related. When expressed in mg/dL, the product of the two ([Ca]×[P]) is 30 ~ 40. When ([Ca]×[P]) > 40, calcium and phosphorus are deposited in bone tissue in the form of bone salt. If ([Ca]×[P]) < 35, it will hinder the calcification of bone, even make the bone salt dissolve, and affect the osteogenic effect. The relative stability of blood calcium and phosphorus content depends on the relative balance of calcium and phosphorus absorption and excretion, calcium and decalcification metabolism. These balances are regulated by hormones such as vitamin D3, parathyroid hormone and calcitonin.

After removing the organic phosphorus from serum, the inorganic phosphorus salt and ammonium molybdate reagent generate phosphomolybdic acid, which is blue after being reduced by ferrous sulfate and has light absorption at 660 nm. In this kit, the phosphorus content in blood is calculated by measuring the absorbance of 660 nm.

Required but not provided:

Centrifuge, transferpettor, spectrophotometer, 1 mL glass cuvette and distilled water.

Procedure:

I. Sample preparation:

1. Serum pretreatment: take 50 μL of serum, add 950 μL of Reagent I, mix well, centrifugate at 8000 rpm for 10 min at room temperature, take supernatant for test.

II. Determination procedure

1. Preheat spectrophotometer for 30 minutes, adjust wavelength to 660 nm, set zero with distilled water.
2. Standard working solution: dilute the standard with distilled water to obtain 0.5 mmol/L standard solution.
3. Add reagents according to the following table.

Reagent(μL)	Blank tube (B)	Standard tube (S)	Test tube (T)
Standard solution	-	250	-
Supernatant	-	-	250
Distilled water	250	-	-
Reagent I	250	250	250
Working solution	500	500	500

After mixing, let it stand for 10 minutes, measure the absorbance at 660 nm, and record it as A_B, A_S, A_T. Standard tube and blank tube only need to be measured once or twice. **The results should be determined within 40 minutes**.

III. Calculation of Blood Phosphorus Concentration

Blood Phosphorus Concentration(mmol/L)= $[C_S \times (A_T - A_B) \div (A_S - A_B)] \times D$

$= 10 \times (A_T - A_B) \div (A_S - A_B)$

C_S: Standard concentration, 0.5 mmol/L;

D: Sample dilution ratio, (50 μL serum + 950 μL Reagent I) ÷ 50 μL serum = 20.

Note:

1. Hemolysis should be avoided as far as possible in the determination process, because the organic phosphate ester in red blood cells can be hydrolyzed by enzymes after entering the serum, which will increase the content of inorganic phosphorus in the serum.
2. When the determination of A is greater than 0.78, it is recommended to dilute supernatant with Reagent I before performing the measurement, and multiply the dilution factor in the calculation formula.

Technical Specifications:

Minimum Detection Limit: 0.0082 mmol/mL

Linear Range: 0.015625-1 mmol/mL

Experimental example:

1. Operate as the procedure with guinea pig serum, A_T=0.671, A_B=0.023, A_S=0.423, calculate:
   Blood Phosphate(mmol/L)= $10 \times (A_T - A_B) \div (A_S - A_B)$=16.2 mmol/L.

Related products:

BC2770/BC2775 Blood Potassium Content Assay Kit
BC2790/BC2795 Blood Magnesium Content Assay Kit

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