Blood Phosphate Content Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Spectrophotometer/Microplate reader

Cat No: NA0426

Size: 100T/96S

Components:

Reagent I: 110 mL×1, store at 4℃.

Reagent II: Powder×2, store at 4℃. Add 1 mL of distilled water for full dissolution, then slowly add 272 μL of concentrated H_2SO_4 before use, mix well.

Reagent III: Powder×2, store at 4℃. Prepare when the solution will be used, add 5.5 mL of distilled water in turn to fully dissolve, then add 0.95 mL of Reagent II to fully mix.

Standard: 1 mL×1, 5 mmol/L inorganic phosphorus, store at 4℃. Dilute 5 times to 1 mmol/L with distilled water before use for standby.

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/microplate reader, micro glass cuvette/96 well flat-bottom

plate and distilled water.

Procedure:

1. Preheat spectrophotometer/microplate reader for 30 minutes, adjust wavelength to 660 nm, set zero with distilled water.

2. 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.

3. Add reagents according to the following table.

Reagent name (μL)	Blank tube (B)	Standard tube (S)	Test tube (T)
Standard solution	-	50	-
Supernatant	-	-	50
Distilled water	50	-	-
Reagent I	50	50	50
Reagent III	100	100	100

After mixing, let it stand for 10 minutes, measure the absorbance at 660 nm, and record it as A_B, A_S, A_T.

Calculation of Blood Phosphorus Concentration

Blood Phosphorus Concentration(mmol/L)= $[C_S \times (A_T - A_B) \div (A_S - A_B)] \times 20$

$$= 20 \times (A_T - A_B) \div (A_S - A_B)$$

C_S: Standard concentration, 1 mmol/L;

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

Note：

1. Reagent III needs to be prepared before use. If it is not used up, it can be stored at 4℃ for up to 3 days.

2. 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.

3. The results are determined within 40 minutes.

Technical Specifications：

Minimum Detection Limit：0.0115 mmol/mL

Linear Range：0.015625-5 mmol/mL

Experimental example:

1. Operate as the procedure with duck blood, A_T=0.806, A_B=0.056, A_S=0.539, calculate: Blood Phosphate(mmol/L)= $20 \times (A_T-A_B) \div (A_S-A_B)$=31.06 mmol/L.

Related products：

NA0669/NA0428 Blood Potassium Content Assay Kit

NA0668/NA0427 Blood Magnesium Content Assay Kit