EZMeta™ Blood Phosphate Colorimetric Assay Kit

Cat #: D-AKC2160

Size: 96T

Storage: Stored at 4°C for 12 months, protected from light

Product Information

Applicable samples: Serum

Assay Principle

Blood phosphate mainly refers to the inorganic phosphate in the blood, which exists in the form of inorganic phosphate salts. After removing the organic phosphate in the serum, the inorganic phosphate salts and ammonium molybdate reagents generate phosphomolybdic acid, which is reduced by ferrous sulfate and turns blue, the phosphate content was determined at 620 nm.

Materials Supplied and Storage Conditions

Kit components	Size	Storage conditions
Reagent Ⅰ	100 mL	4°C
Reagent Ⅱ	1.6 mL	4°C, protected from light
Reagent Ⅲ	1	4°C
Standard	2 mL	4°C

Materials Required but Not Supplied

·Microplate reader or visible spectrophotometer capable of measuring absorbance at 620 nm

·96-well plate or microglass cuvette, precision pipettes, disposable pipette tips

·Centrifuge

·Deionized water

Reagent Preparation

Reagent I: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

Reagent II: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C, protected from light.

Working Reagent III: Prepare before use, add 11 mL of deionized water to dissolve thoroughly. Then add Reagent II and mix thoroughly, and use on the same day; Store at 4°C.

Standard: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

Sample Preparation

1.Serum sample: Take 50 µL serum, add 950 µL Reagent I, mix well and centrifuge 8,000 rpm for 10 min at room temperature. Take the supernatant for testing.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 620 nm, visible spectrophotometer was returned to zero with deionized water.

2. Operation table (the following operations are performed in 96-well plate or microglass cuvette).

Reagent	Blank Well (µL)	Standard Well (µL)	Test Well (µL)
Supernatant	0	0	50
Deionized Water	50	0	0
Standard	0	50	0
Reagent Ⅰ	50	50	50
Working ReagentⅢ	100	100	100

3. Mix well and let stand at room temperature for 10 min, then measure the absorbance at 620 nm. The absorbance of blank well, standard well, test well recorded as A_{Blank}, $A_{Standard}$ and A_{Test}. Finally, calculate $\Delta A_{Test}=A_{Test}-A_{Blank}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

Note: Blank well and standard well only need to measure 1 time. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. Increase the sample quantity appropriately. If ΔA_{Test} is greater than 1.0, the sample can be appropriately diluted with Reagent Ⅰ, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

Blood phosphate concentration calculation:

Blood phosphate content (mmol/dL)=($C_{Standard}\times\Delta A_{Test}\div\Delta A_{Standard}$)×Sample dilution ratio=**$2\times\Delta A_{Test}\div\Delta A_{Standard}$**

$C_{Standard}$: 0.1 mmol/dL; Sample dilution ratio: (50 μL serum+950 μL Reagent Ⅰ)÷50 μL serum=20.

Typical Data

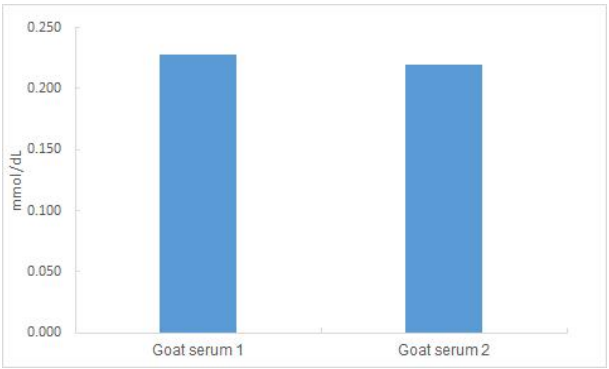

Figure1. The content of goat blood phosphate

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.