



## CheKine™ Micro Blood Phosphate Assay Kit

Cat #: KTB2160

Size: 96 T

	<b>Micro Blood Phosphate Assay Kit</b>		
<b>REF</b>	<b>Cat #:</b> KTB2160	<b>LOT</b>	<b>Lot #:</b> Refer to product label
	<b>Applicable:</b> Serum		
	<b>Storage:</b> Stored at 4°C for 12 months, protected from light		

### 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  $\mu\text{L}$  serum, add 950  $\mu\text{L}$  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 ( $\mu\text{L}$ )	Standard Well ( $\mu\text{L}$ )	Test Well ( $\mu\text{L}$ )
Supernatant	0	0	50
Deionized Water	50	0	0
Standard	0	50	0
Reagent I	50	50	50
Working Reagent III	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  $A_{\text{Blank}}$ ,  $A_{\text{Standard}}$  and  $A_{\text{Test}}$ . Finally, calculate  $\Delta A_{\text{Test}} = A_{\text{Test}} - A_{\text{Blank}}$ ,  $\Delta A_{\text{Standard}} = A_{\text{Standard}} - A_{\text{Blank}}$ .

**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  $\Delta A_{\text{Test}}$  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:

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

$C_{\text{Standard}}$ : 0.1 mmol/dL; Sample dilution ratio:  $(50 \mu\text{L serum} + 950 \mu\text{L Reagent I}) \div 50 \mu\text{L serum} = 20$ .

## Typical Data

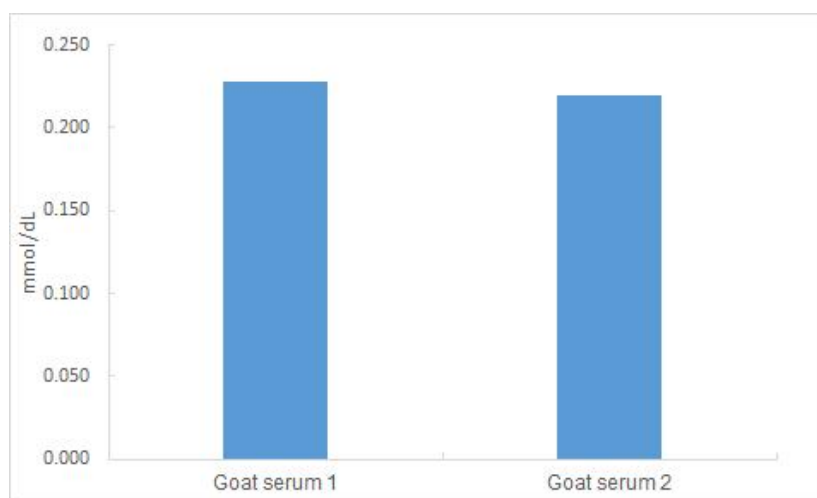


Figure1. The content of goat blood phosphate

## Precautions

1. Working Reagent III needs to be prepared before use, and is limited to use on the same day.
2. During the measurement process, hemolysis should be avoided, as organic phosphate esters in red blood cells can be hydrolyzed by enzymes after entering the serum, resulting in an increase in serum inorganic phosphate content.

## Recommended Products

Catalog No.	Product Name
KTB4011	CheKine™ Micro Soil Phosphate(S-PHOS) Assay Kit
KTB3050	CheKine™ Micro Water and Soil Nitrite Content Assay Kit

## Disclaimer

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