PRODUCT INFORMATION & MANUAL

Phosphate Assay Kit (Colorimetric)

NBP3-24530

For research use only.
Not for diagnostic or therapeutic procedures.

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Phosphate Assay Kit
(Colorimetric)

Catalog No: NBP3-24530

Method: Colorimetric method

Specification: 96T (Can detect 80 samples without duplication)

Instrument: Microplate reader

Sensitivity: 0.001 mmol/L

Detection range: 0.001 -0.20 mmol/L

Average intra-assay CV (%): 4

Average inter-assay CV (%): 8

Average recovery rate (%): 98

- ▲ This kit is for research use only.
- ▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
- ▲ Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

This kit can be used to measure phosphate content in serum, plasma, tissue and cell samples.

▲Detection principle

Phosphorus is an important mineral, one of the essential nutrients for all animals, and is essential for maintaining phosphate homeostasis.

Malachite green method is a sensitive method for phosphate analysis. The principle is that the compounds formed by phosphoric acid and molybdate under acidic conditions will form color substances with malachite green. The depth of color is proportional to the content of phosphate. The phosphorus content can be calculated indirectly be measuring the OD value at 636 nm.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Chromogenic Agent A	12 mL×1 vial	2-8℃ , 12 months, shading light
Reagent 2	Chromogenic Agent B	4 mL×1 vial	2-8℃ , 12 months
Reagent 3	Protein Precipitator	60 mL×1 vial	2-8℃ , 12 months
Reagent 4	10 mmol/L Standard Solution	1 mL × 1 vial	2-8℃ , 12 months
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other.			

▲Materials prepared by users

Instruments

Micropipettor, Incubator, Vortex mixer, Centrifuge, Microplate reader (630-650 nm)

Reagents:

Normal saline (0.9% NaCl)

⚠ Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

⚠ Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲ The key points of the assay

1. When the absoluted OD value is more than 1.2, it is necessary to increase the dilution ratio and detect again.
2. Incubate reagent 1 at 37°C until no gelatinous substance before use.
3. Avoid the contamination of phosphorus, it is recommended to wash the experimental equipment for several times (about 10 times).

Pre-assay preparation

▲ Reagent preparation

1. Bring all reagents to room temperature before use.
2. Preparation of chromogenic working solution:

   Prepare the chromogenic working solution according to the ratio of reagent 1: reagent 2: double distilled water =3: 1: 4 (mix fully). Incubate the prepared working solution at 37°C for 1 h. Prepare the fresh solution before use and the prepared solution should be used up on the same day.
3. Preparation of 0.2 mmol/L standard:

   Dilute reagent 4 with double distilled water at the ratio of 1: 49. Prepare the fresh needed amount before use and prepared solution can be stored at 2-8°C for 7 days.

▲ Sample preparation

1. Serum and plasma samples:

Mix sample with reagent 3 at the ratio of 1:1. Centrifuge at 4°C at 12000 g for 10 min. Take the supernatant for detection.

2. 10% tissue homogenate sample:

Accurately weigh the tissue sample, add 9 times the volume of normal saline according to the ratio of Weight (g): Volume (mL) =1:9. Mechanical homogenate the sample in ice water bath. Centrifuge at 4°C at 12000 g for 10 min, then take the supernatant. Mix supernatant with reagent 3 at the ratio of 1:1. Centrifuge at 4°C at 12000 g for 10 min. Take the supernatant for detection.

3. Cell sample:

Collect the cells and wash the cells with PBS (0.01 M, pH 7.4) for 1~2 times. Centrifuge at 1000 g for 10 min and then discard the supernatant and keep the cell sediment. Add homogenization medium at a ratio of cell number (10^6): normal saline (mL) =5: 1. Sonicate or grind wit h hand-operated in ice water bath. Centrifuge at 4°C at 12000 g for 10 min, then take the supernatant. Mix supernatant with reagent 3 at the ratio of 1:1. Centrifuge at 4°C at 12000 g for 10 min. Take the supernatant for detection.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.001-0.20 mmol/L).

The recommended dilution factor for different samples is as follows (for reference only)

Sample type	Dilution factor
10% Rat liver tissue homogenate	15-25
10% Rat heart tissue homogenate	15-25
10% Rat spleen tissue homogenate	15-25
10% Rat brain tissue homogenate	15-25
10% Rat kidney tissue homogenate	15-25
10% Mouse heart tissue homogenate	15-25
10% Mouse lung tissue homogenate	15-25
10% Mouse ovarian tissue homogenate	50-70
Rat serum	8-12
Rat plasma	8-12
Human plasma	8-12
10^6 HL-40 cell	2-5

Note: The diluent is normal saline (0.9% NaCl).

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

Note: A-H, standard wells; S1-S80, sample wells.

▲Detailed operation steps

1. The preparation of standard curve

Dilute 0.2 mmol/L standard with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0.20, 0.15, 0.12, 0.10, 0.05, 0.02, 0.01, 0 mmol/L.. Reference is as follows:

Number	Standard concentrations (mmol/L)	0.2 mmol/L standard (μL)	Double distilled water (μL)
A	0	0	200
B	0.01	10	190
C	0.02	20	180
D	0.05	50	150
E	0.10	100	100
F	0.12	120	80
G	0.15	150	50
H	0.20	200	0

2. The measurement of samples

(1) Standard well: Take 20 μL of standard solution with different concentration to the well.

Sample well: Take 20 μL of sample to the well.

(2) Add 200 μL of chromogenic working solution to each well.

(3) Mix fully with microplate reader for 5 s and incubate at 37°C for 20 min with shading light.

(4) Measure the OD value of each well at 636 nm with microplate reader.

▲Summary operation table

	Standard well	Sample well
Standard solution with different concentration (μL)	20	
Sample (μL)		20
Chromogenic working solution (μL)	200	200
Mix fully and incubate at 37°C for 20 min with shading light. Measure the OD value of each well.		

▲Calculation

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample. The standard curve is: y= ax + b.

1. For serum/plasma samples :

$$\text{Phosphate (mmol/L)} = (\Delta A - b) \div a \times f \times 2^*$$

2. For tissue sample:

$$\text{Phosphate (mmol/kg wet weight)} = (\Delta A - b) \div a \div (m \div V) \times f \times 2^*$$

3. For cell sample:

$$\text{Phosphate (mmol/10^6)} = (\Delta A - b) \div a \div (n \div V) \times f \times 2^*$$

Note:

y: $OD_{Standard}$ – OD_{Blank} (OD_{Blank} is the OD value when the standard concentration is 0).

x: The concentration of standard.

a: The slope of standard curve.

b: The intercept of standard curve.

ΔA: OD_{Sample} – OD_{Blank}.

m: The weight of tissue sample, g.

V: The volume of normal saline in the preparation step of sample, mL.

n: The number of cell sample/10^6.

2*: Dilution factor of sample in preparation of supernatant.

f: Dilution factor of sample before test.

Appendix I Data

▲Example analysis

For rat liver tissue, take 10% rat liver tissue homogenate, dilute for 20 times and carry the assay according to the operation table.

The results are as follows:

standard curve: y = 5.1343 x - 0.0073, the average OD value of the blank is 0.142, the average OD value of the sample is 0.564, and the calculation result is:

Phosphate content (mmol/kg wet weight) = (0.564 – 0.142 + 0.0073) ÷ 5.1342 ÷ (0.1 ÷ 0.9) × 20 × 2 = 30.06 mmol/kg wet weight