



PRODUCT INFORMATION & MANUAL

Alkaline Phosphatase Activity Assay Kit (Colorimetric) *NBP3-24464*

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

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Alkaline Phosphatase Activity Assay Kit (Colorimetric)

Catalog No: NBP3-24464

Method: Colorimetric method

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

Measuring instrument: Microplate reader

Sensitivity: 0.13 King unit/100 mL

Detection range: 0.13-50 King unit/100 mL

Average intra-assay CV (%): 5.1

Average inter-assay CV (%): 8.5

Average recovery rate (%): 94

- ▲ 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 alkaline phosphatase (ALP) activity in serum (plasma), tissue, cells and other samples.

▲ Background

Alkaline phosphatase (ALP) is a group of cytomembrane-related enzymes with hydrolysis and transfer activity, acting on a variety of phosphate substrates. ALP is a homologous dimerase and each catalytic site contains three metal ions. There are four isozymes in humans: tissue nonspecific ALP, intestinal ALP, placental ALP and genital cell ALP.

▲ Detection principle

Alkaline phosphatase decompose benzene disodium phosphate to produce free phenol and phosphoric acid. Phenol react with 4-aminopyrline in alkaline solution and oxidizes with potassium ferricyanide to form red quinone derivative. The enzyme activity can be calculated indirectly by measuring the OD value.

▲ Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Buffer Solution	3 mL × 1 vial	2-8°C , 12 months, shading light
Reagent 2	Substrate Solution	3 mL × 1 vial	2-8°C , 12 months, shading light
Reagent 3	Chromogenic Agent	18 mL × 1 vial	2-8°C , 12 months, shading light
Reagent 4	0.5 mg/mL Phenol Standard	1.5 mL × 1 vial	2-8°C , 12 months, shading light
	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

Microplate reader (500-530 nm), Micropipettor, Centrifuge, Incubator, Vortex mixer

Reagents

Double distilled water, Normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4)

▲ 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.

Pre-assay preparation

▲ Reagent preparation

The preparation of working solution

Mix the reagent 1 and reagent 2 at the ratio of 1:1 fully. Prepare the fresh solution before use and the unused solution can be stored at 2-8°C with shading light for 24 hours.

▲ Sample preparation

The samples should be prepared as conventional methods. Also please refer to appendix II.

Sample requirements

1. Samples should not contain EDTA, citrate, oxalate, high concentration of inorganic phosphorus.
2. Glucose, amino sugar, benzidine in the sample will inhibit the activity of ALP.

▲ 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 (0.13-50 King unit/100 mL).

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

Sample type	Dilution factor
Human plasma	1
Human urine	1
Rat serum	1
Cells culture supernatant	1
10% Mouse kidney tissue homogenate	30-50
10% Mouse liver tissue homogenate	1
10% Mouse brain tissue homogenate	1
HepG2 cells	1

Note: The diluent is normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4).

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

The preparation of standard curve

Dilute 0.5 mg/mL phenol standard with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 0.025, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5 mg/mL. Reference is as follows:

Number	Standard concentrations (mg/mL)	0.5 mg/mL phenol standard (μL)	Double distilled water (μL)
A	0	0	100
B	0.025	5	95
C	0.05	10	90
D	0.1	20	80
E	0.2	40	60
F	0.3	60	40
G	0.4	80	20
H	0.5	100	0

The measurement of samples

1. **Standard well:** add 5 μL of standards with different concentrations to the corresponding wells.
Sample well: add 5 μL of sample to the corresponding wells.
2. Add 50 μL of working solution and mix fully for 30 s with microplate reader.
3. Incubate at 37°C for 15 min, then add 150 μL of reagent 3 immediately, mix fully.
4. Measure the OD values of each well at 520 nm with microplate reader.

▲ Summary operation table

	Standard well	Sample well
Standards with different concentrations (μL)	5	
Sample (μL)		5
Working solution (μL)	50	50
Mix fully and incubate at 37°C for 15 min.		
Reagent 3 (μL)	150	150
Mix fully and measure the OD values 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. Serum (plasma) and other liquid sample

Definition: The amount of 1 mg phenol produced by 100 mL sample react with the substrate in 15 min is defined as 1 ALP activity unit.

$$\text{ALP activity (King unit/100 mL)} = (\Delta A - b) \div a \times V_1 \times f$$

2. Tissue and cells sample

Definition: The amount of 1 mg phenol produced by 1 g tissue protein react with the substrate in 15 min is defined as 1 ALP activity unit.

$$\text{ALP activity (King unit/gprot)} = (\Delta A - b) \div a \div C_{pr} \times f$$

Note:

y: The absolute OD value of standard;

x: The concentration of standard;

a: The slope of standard curve;

b: The intercept of standard curve.

ΔA : Absolute OD ($OD_{\text{Sample}} - OD_{\text{Blank}}$).

V_1 : The volume of sample in definition, 100 mL.

f: Dilution factor of sample before test.

C_{pr} : Concentration of protein in sample, gprot/mL

Appendix I Data

▲ Example analysis

Take 5 µL of rat serum and carry the assay according to the operation table.

The results are as follows:

Standard curve: $y = 3.059x + 0.0027$, the average OD value of the sample well is 0.422, the average OD value of the blank well is 0.091, the concentration of protein in sample is 9.23gprot/L, and the calculation result is:

$$\text{ALP activity (King unit/100 mL)} = \frac{0.422 - 0.091 - 0.0027}{3.059} \times 100 = 10.73 \text{ King unit/ 100mL}$$

Appendix II Sample preparation

The following sample pretreatment methods are for reference only.

▲ Serum

Collect fresh blood and stand at 25°C for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4°C . Take the serum (which is the upper light yellow clarified liquid layer) to preserve it on ice for detection. If not detected on the same day, the serum can be stored at -80°C for a month.

▲ Plasma

Take fresh blood into the tube which has anticoagulant (heparin is recommended), centrifuge at 700-1000 g for 10 min at 4°C . Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) to preserve it on ice for detection. If not detected on the same day, the plasma can be stored at -80°C for a month.

▲ Urine

Collect fresh urine and centrifuge at 10000 g for 15 min at 4°C . Take the supernatant to preserve it on ice for detection. If not detected on the same day, the urine can be stored at -80°C for a month.

▲ Tissue sample

Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2 - 8 °C . Absorb the water with filter paper and weigh. Homogenize at the ratio of the volume of homogenized medium (2-8°C) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4 °C . Take the supernatant to preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80°C for a month.

▲ Cells

Collect the cells (do not use trypsin and EDTA) 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): homogenization medium (μL) = 1: 300-500. Sonicate or grind with hand-operated in ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant. If not detected on the same day, the cells sample (without homogenization) can be stored at -80°C for a month.

Note:

1. Homogenized medium: Normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4).

2. Homogenized method:

(1) Hand-operated: Weigh the tissue and mince to small pieces (1 mm^3), then put the tissues pieces to glass homogenized tube. Add homogenized medium into homogenized tube, place the tube into the ice bath with left hand, and insert the glass tamping rod vertically into the homogenized tube with the right hand to grind up and down for 6-8 min.

Or put the tissue into the mortar, and add liquid nitrogen to grind fully. Then add the homogenized medium to homogenize.

(2) Mechanical homogenate: Weigh the tissue to EP tube, add the homogenized medium to homogenize the tissue with homogenizer instrument (60 Hz, 90s) in the ice bath. (For samples of skin, muscle and plant tissue, the time of homogenization can be properly prolonged.)

(3) Ultrasonication: Treat the cells with ultrasonic cell disruptor (200 W, 2 s/ time, interval for 3 s, the total time is 5 min).

Appendix III References

1. Millán J L. Alkaline Phosphatases. *Biochemical Society Transactions*, 1990, 18(2):178-180.
2. Millán J L. Alkaline Phosphatases: Structure, substrate specificity and functional relatedness to other members of a large superfamily of enzymes. *Purinergic Signalling*, 2009, 2(2): 335-341.
3. Fernandez N J, Kidney B A. Alkaline phosphatase: beyond the liver. *Veterinary Clinical Pathology*, 2010, 36(3): 223-233.