PRODUCT INFORMATION & MANUAL

Alkaline Phosphatase Activity Assay Kit (Colorimetric)

NBP3-24466

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

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Alkaline Phosphatase Activity Assay Kit (Colorimetric)

Catalog No: NBP3-24466

Method: Colorimetric method

Specification: 96T (Can detect 79 samples without duplication)

Measuring instrument: Microplate reader

Sensitivity: 0.27 U/L

Detection range: 0.27-50.8 U/L

Average intra-assay CV (%): 1.2

Average inter-assay CV (%): 4.6

- ▲ 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 animal tissue, serum (plasma) and cell 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

Under alkaline conditions, alkaline phosphatase catalyzes the hydrolysis of p-nitrobenzene phosphate disodium to produce p-nitrophenol and phosphoric acid. Under strong alkaline conditions, p-nitrophenol is bright yellow and has a maximum absorption peak at 405 nm. Therefore, the activity of ALP can be calculated by measuring the OD value at 405 nm.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Buffer Solution	60 mL×1 vial	-20℃ , 12 months
Reagent 2	Substrate	Powder×2 vials	-20℃ , 12 months, shading light
Reagent 3	10 mmol/L p-Nitrophenol Standard Solution	0. 4mL×1 vial	-20℃ , 12 months, shading light
Reagent 4	Stop Solution	12 mL×1 vial	-20℃ , 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

Microplate reader (400-415 nm), Micropipettor, Centrifuge, Incubator

▲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

Reagent 2 working solution and standard should be stored with shading light.

Pre-assay preparation

▲Reagent preparation

1. Bring all reagents to room temperature before use.
2. The preparation of reagent 2 working solution:

   Dissolve reagent 2 with 3 mL of reagent 1. The prepared solution can be stored at -20℃ with shading light for 24 hours.

3. The preparation of 500 μmol/L standard:

   Mix the reagent 3 and reagent 1 at the ratio of 1:19. Prepare the fresh needed amount before use and store it with shading light for detection.

▲Sample preparation

1. Serum (Plasma): Detect the sample directly.
2. Tissue sample: Weigh the tissue accurately. Add normal saline (0.9% NaCl) in a weight (g): volume (mL) ratio of 1: 9, homogenize mechanically in ice water bath to break cells fully. Then centrifuge at 10000 g for 10 min at 4℃ and collect the supernatant for measurement. Meanwhile, determine the protein concentration of supernatant.
3. Cell sample: Add normal saline (0.9% NaCl)at a ratio of cell number ($3*10^6$): 0.9% NaCl (μL) =1: 200-400.
4. Sample requirements: Sample solution should avoid inhibitors of alkaline phosphatase, such as EDTA, fluoride ion and citrate.

▲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 (0.27-50.8 U/L).

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

Sample type	Dilution factor
Human serum	10-20
Mouse serum	20-30
Rat serum	10-20
Mouse plasma	10-20
HepG2 supernatant	8-12
10% Rat kidney tissue homogenate	400-1200
10% Mouse liver tissue homogenate	10-15
10% Rat lung tissue homogenate	50-100
10% Mouse brain tissue homogenate	30-50
Human urine	1

Note: The diluent is reagent 1.

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S0	S8	S16	S24	S32	S40	S48	S56	S64	S72
B	B	B	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
C	C	C	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
D	D	D	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
E	E	E	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
F	F	F	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
G	G	G	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
H	H	H	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79

Note: A-H, standard wells; S0, control wells; S1-S79, sample wells.

▲Detailed operation steps

The preparation of standard curve

Dilute 500 μmol/L standard solution with reagent 1 to a serial concentration. The recommended dilution gradient is as follows: 500, 400, 320, 240, 160, 80, 40, 0 μmol/L. Reference is as follows:

Number	Standard concentrations (μmol/L)	500 μmol/L standard solution (μL)	Reagent 1 (μL)
A	0	0	200
B	40	16	184
C	80	32	168
D	160	64	136
E	240	96	104
F	320	128	72
G	400	160	40
H	500	200	0

The measurement of samples

1. Standard well: Take 50 μL of standards with different concentrations into the standard wells.
   Sample well: Add 50 μL of sample into the sample wells.
   Control well: Add 50 μL of reagent 1 into the control wells.
2. Add 50 μL of reagent 2 working solution into the control wells and sample wells.
   Add 50 μL of reagent 1 into the standard wells.
3. Incubate at 37℃ for 10 min.
4. Add 100 μL of reagent 4 into each well.
5. Mix fully with microplate reader for 5 s. Measure the OD values of each well at 405 nm with microplate reader.

▲Summary operation table

	Standard well	Sample well	Control well
Standards with different concentrations (μL)	50		
Sample (μL)		50	
Reagent 1 (μL)	50		50
Reagent 2 Working solution (μL)		50	50
Incubate at 37℃ for 10 min.			
Reagent 4 (μL)	100	100	100
Mix fully. 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) sample

Definition: The amount of 1 μmol p-nitrophenol produced by 1 L serum (plasma) per minute catalyze the substrate at 37℃ is defined as 1 activity unit.

$$\text{ALP activity (U/L)} = (\Delta A - b) \div a \div T \times f$$

2. Tissue sample

Definition: The amount of 1 μmol p-nitrophenol produced by 1 g tissue protein per minute catalyze the substrate at 37℃ is defined as 1 activity unit.

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

3. Cell sample

Definition: The amount of 1 μmol p-nitrophenol produced by 1 g cell protein per minute catalyze the substrate at 37℃ is defined as 1 activity unit.

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

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} – $OD_{Control;}$

T: Reaction time, 10 min;

f: Dilution factor of sample before test;

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

Appendix I Data

▲Example analysis

For human serum, dilute human serum with reagent 1 for 10 times, take 50 μL of diluted samples and carry the assay according to the operation table. The results are as follows:

Standard curve: y = 0.0027 x – 0.0051, the OD value of the control is 0.099, the OD value of the sample is 0.544, and the calculation result is:

ALP activity (U/L)= (0.544-0.099+0.0051) ÷ 0.0027÷10 ×10=166.70 U/L

Appendix II 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.