Alkaline Phosphatase (ALP) Activity Assay Kit

Catalog Number EEA002 (96 tests)

Rev 2.0

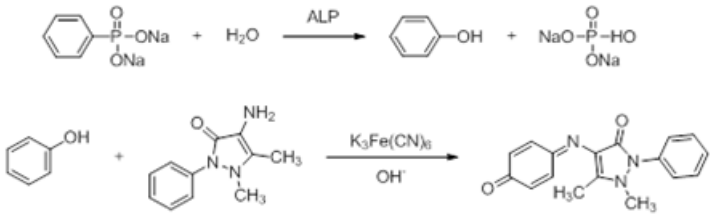
For safety and biohazard guidelines, read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

This kit can be used to measure alkaline phosphatase (ALP) activity in serum, plasma, tissue, cells and other samples.

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 where each catalytic site contains three metal ions. There are four isozymes in humans: tissue nonspecific ALP, intestinal ALP, placental ALP and genital cell ALP.

Alkaline phosphatase decomposes 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 a red quinone derivative. The enzyme activity can be calculated indirectly by measuring the OD value.



Contents and storage

Kit and components are shipped at 2-8 ℃. An unopened kit can be stored at 2-8 ℃ for 12 months.

Components	Quantity (96 tests)
Buffer Solution	3 mL
Substrate Solution	3 mL
Chromogenic Agent	18 mL
0.5 mg/mL Phenol Standard	1.5 mL
Microplate	1 plate
Plate Sealer	2 pieces

Required materials

- Distilled or deionized water
- Normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4)
- Microtiter plate reader with software capable of measurement at or near 520 nm
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solutions.
- Incubator capable of maintaining 37 ℃.

Procedural guidelines

IMPORTANT! Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.

Sample preparation guidelines

Sample requirements

- Samples should not contain EDTA, citrate, oxalate, high concentration of inorganic phosphorus.
- The presence of glucose, amino sugars, or benzidine in the samples will inhibit the activity of ALP.

Serum and plasma samples: Detect the sample directly. If the sample is turbid, centrifuge at 10000 g for 10 min at 4 ℃, then take the supernatant for detection.

Urine: Collect fresh urine and centrifuge at 10000 g for 15 min at 4 ℃. Take the supernatant to preserve it on ice for detection.

Tissue sample:

- Take 0.02-1 g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8℃ to remove blood cells.
- Absorb the water with filter paper and weigh.
- Homogenize at the ratio of the volume of PBS (0.01 M, pH 7.4)(mL):the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4℃.
- Take the supernatant and preserve it on ice for detection.

Meanwhile, determine the protein concentration of supernatant (Catalog No. 23227).

If not detected on the same day, the tissue sample (without homogenization) can be stored at -80℃ for 1 month.

Cells:

- Collect the cells (do not use trypsin with EDTA) and wash the cells with PBS (0.01 M, pH 7.4) for 1 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): PBS (0.01 M, pH 7.4)(μL) =1: 300-500.
- Sonicate or grind with hand-operated in ice water bath.
- Centrifuge at 10000 g for 10 min at 4℃, then take the supernatant and preserve it on

ice for detection.
Meanwhile, determine the protein concentration of supernatant (Catalog No. 23227).
If not detected on the same day, the cells sample (without homogenization) can be stored at -80℃ for 1 month.

Prepare samples

It is recommended to take 2~3 samples with expected large difference to do a 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).
Note: Use all samples within 2 hours of dilution
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) or PBS (0.01 M, pH 7.4).

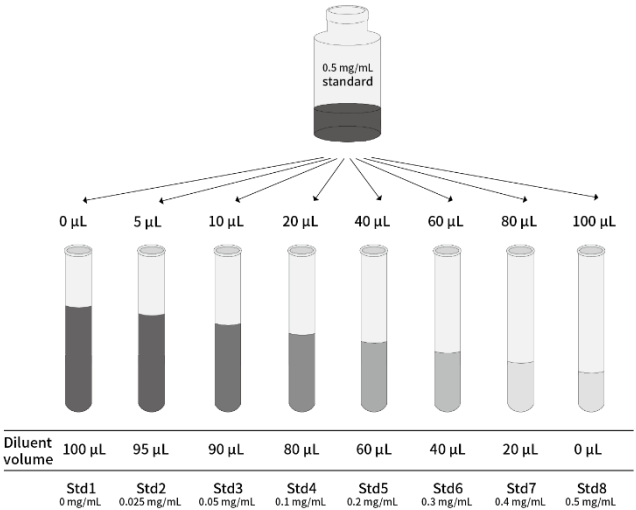
The preparation of working solution

Mix 3 mL buffer solution with 3 mL of substrate solution. Prepare the solution fresh before use. The unused solution can be stored at 2-8 ℃ in the dark, covered, for 24 hours.

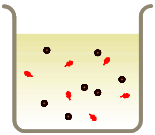
Prepare diluted standards

Note: Use glass or plastic tubes for diluting standards.

Dilute 0.5 mg/mL phenol standard with deionized or 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.



Assay procedure



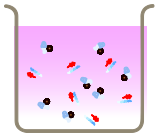
1. Add sample and standard

a. **Standard wells:** Add 5 μL of standards with different concentrations to the corresponding wells.

b. **Sample wells:** Add 5 μL of sample to the corresponding wells.

2. Add substrate

a. Add 50 μL of working solution and mix fully for 30 s with microplate reader.



b. Incubate at 37 ℃ for 15 min, then add 150 μL Chromogenic Agent immediately, mix fully.

c. Measure the OD values of each well at 520 nm with microplate reader.

Calculation

1. Average the duplicate reading for each standard.
2. Subtract the mean OD value of the blank (Std1) from all standard readings. This is the absoluted OD value.
3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (y = ax + b) with graph software (or EXCEL).

Serum (plasma) and other liquid sample:

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

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

Tissue and cells sample:

Definition: The amount of 1 mg phenol produced by 1 g tissue protein reacted with the substrate in 15 min is defined as 1 King unit/100 mL.

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

[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 ($OD_{Sample} - OD_{Blank}$).

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

To easy calculate the test results, refer to the calculation file available on the webpage.

Example analysis

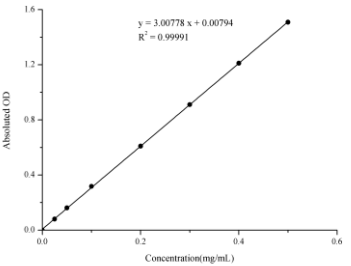
Take 5 μL of rat serum and carry the assay according to the operation table. The results are as follows: Standard curve: y = 3.059 x + 0.0027, the average OD value of the sample well is 0.422, the average OD value of the blank well is 0.091, and the calculation result is:

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

Performance characteristics

▪Standard curve (example)

The following data were obtained for the various standards over the range of 0–0.5 mg/mL standard.

Concentration (mg/mL)	OD at 520 nm	Standard Curve
0.50	1.563	
0.40	1.265	
0.30	0.965	
0.20	0.664	
0.10	0.371	
0.05	0.215	
0.03	0.135	
0.00	0.054	

Inter-assay Precision

Three human serum samples were assayed 17 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (King unit/100 mL)	5.08	29.94	61.18
%CV	8.9	10.6	6.0

CV = Coefficient of Variation

▪Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (King unit/100 mL)	1.00	2.00	4.00
%CV	7.3	4.8	3.2

CV = Coefficient of Variation

▪Expected values

This assay was tested with human serum, and rats plasma samples without dilutions.

Sample	Range (King unit/100 mL)	Average (King unit/100 mL)
Human Serum	5-13	11
Rats plasma	2-8	4.03

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 94%.

	Sample 1 (low conc.)	Sample 2 (middle conc.)	Sample 3 (high conc.)
Expected Conc. (King unit/100 mL)	10.0	30.0	60.0
Observed Conc. (King unit/100 mL)	8.40	28.50	62
Recovery rate (%)	84	95	104

- Recommended 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												

▪Sensitivity

The analytical sensitivity of the assay is 0.13 King unit/100 mL. This was determined by adding two standard deviations to the mean OD obtained when the zero standard was assayed 20 times and calculating the corresponding concentration.

Limited product warranty

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