Technical Manual

Alkaline Phosphatase (ALP) Activity Assay Kit

- Catalogue Code: MAES0080
- Size: 96T
- Research Use Only

1. Key features and Sample Types

Detection method:

Colorimetric method

Specification:

96T

Range:

0.13-50 King unit/100 mL

Sensitivity:

0.13 King unit/100 mL

Storage:

2-8°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

2. 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

3. Intended Use

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

4. 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.

5. Kit components & storage

Item	Specification	Storage
Buffer Solution	1.5 mL × 2 vials	2-8°C, 6 months, shading light
Substrate Solution	1.5 mL × 2 vials	2-8°C, 6 months, shading light
Chromogenic Agent	9 mL × 2 vials	2-8°C, 6 months, shading light
Phenol Standard (0.5 mg/mL)	1.5 mL × 1 vial	2-8°C, 6 months, shading light
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Microplate Reader (500-530 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- Normal Saline (0.9% NaCl)
- PBS (0.01 M, pH 7.4)

6. Assay Notes:

After incubating at 37°C for 15 min, quickly add chromogenic agent.

7. Reagent preparation:

1. Bring all reagents to room temperature before use.

2. The preparation of **working solution:** Mix the buffer solution and substrate solution 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.

8. Sample Preparation

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

1. Serum sample:
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.

2. Plasma sample:
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.

3. 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.

4. 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 (2×10^6): Normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4) (μL) =1: 300. Sonicate the sample on an ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection. If not detected on the same day, the cells sample (without homogenization) can be stored at -80°C for a month.

5. 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 Normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4) (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. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80°C for a month.

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. 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
0% 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);

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 520 nm

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.

10. Operation Steps

The preparation of standard curve

Dilute phenol standard (0.5 mg/mL) 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.

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 chromogenic agent immediately, mix fully.

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

Operation Table

	Standard well	Sample well
Standards with different concentrations (μL)	5	
Sample (μL)		5
Working solution (μL)	50	50
Mix fully for 30 s with microplate reader and incubate at 37°C for 15 min.		
Chromogenic agent (μL)	150	150
Mix fully and measure the OD values of each well at 520 nm with microplate reader.		

11. Calculations

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

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

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: $OD_{Sample} - OD_{Blank}$
V_1: The volume of sample in definition, 100 mL.
f: Dilution factor of sample before test.
C_{pr}: Concentration of protein in sample, mgprot/mL.

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.

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

12. Performance Characteristics

Detection Range	0.13-50 King unit/100 mL
Sensitivity	0.13 King unit/100 mL
Average recovery rate (%)	94
Average inter-assay CV (%)	8.5
Average intra-assay CV (%)	5.1

Analysis

Take 5 μL of rat serum, 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, the concentration of protein in sample is 9.23 gprot/L, and the calculation result is:

ALP activity (King unit/100 mL) = (0.422 - 0.091 - 0.0027) ÷ 3.059 × 100 = 10.73 King unit/100 mL

Safety Notes

Some of the reagents in the kit contain dangerous substances. Avoid touching 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.

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

Email: info@assaygenie.com **Web:** www.assaygenie.com

Notes:

Email: info@assaygenie.com **Web:** www.assaygenie.com

Notes:

Email: info@assaygenie.com **Web:** www.assaygenie.com

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Contact Details

Email: info@assaygenie.com

Web: www.assayenie.com