TECHNICAL MANUAL

Alkaline Phosphatase (ALP) Activity Assay Kit

- **SKU CODE:** MAES0080
- **SIZE:** 96 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

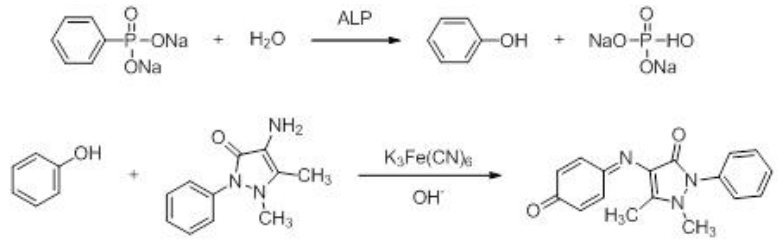
- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add standards and samples
- Incubate with working solution
- Add chromogenic agent
- Measure OD values

2. Intended use

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

3. Detection principle

Alkaline phosphatase decomposes disodium phenyl phosphate to produce free phenol and phosphoric acid. Phenol reacts with 4-aminoantipyrine 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.



4. Kit components & storage

Item	Component	Size 1 (96 T)	Size 2 (500 Assays)	Storage
Reagent 1	Buffer Solution	3 mL × 1 vial	15 mL × 1 vial	2-8 °C, 12 months, protect from light
Reagent 2	Substrate Solution	3 mL × 1 vial	15 mL × 1 vial	2-8 °C, 12 months, protect from light
Reagent 3	Chromogenic Agent	18 mL × 1 vial	50 mL × 2 vials	2-8 °C, 12 months, protect from light

Item	Component	Size 1 (96 T)	Size 2 (500 Assays)	Storage
Reagent 4	0.5 mg/mL Phenol Standard	1.5 mL × 1 vial	5 mL × 1 vial	2-8 °C, 12 months, protect from light
	Microplate	96 wells	/	No requirement
	Plate Sealer	2 pieces		

5. 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)

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of working solution:** For each well, prepare 50 µL of working solution by mixing 25 µL of buffer solution and 25 µL of substrate solution. Mix well. Store at 2-8 °C for 1 day protected from light.
3. **Preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 0.5 mg/mL phenol standard with double distilled water to serial concentrations. The recommended dilution gradient is as follows: 0, 0.025, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5 mg/mL.

7. Sample preparation

Sample preparation:

Serum and plasma: Detect directly. If not detected on the same day, the serum or plasma can be stored at -80 °C for a month.

Tissue sample:

1. Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

2. Wash tissue in cold PBS (0.01 M, pH 7.4).

3. Homogenize 20 mg tissue in 180 µL normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4) with a dounce homogenizer at 4 °C.

4. Centrifuge at 10,000 × g for 10 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection.

5. Meanwhile, determine the protein concentration of supernatant.

Cell (adherent or suspension) samples:

1. Harvest the number of cells needed for each assay (initial recommendation 1 × 10^6 cells).

2. Wash cells with PBS (0.01 M, pH 7.4).

3. Homogenize 1 × 10^6 cells in 300-500 µL normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4) with an ultrasonic cell disruptor at 4 °C.

4. Centrifuge at 10,000 × g for 10 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection.

5. Meanwhile, determine the protein concentration of supernatant.

Dilution of samples:

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

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). For the dilution of other sample types, please perform a pretest to confirm the dilution factor.

8. Operating steps

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 seconds with microplate reader.
3. Incubate at 37 °C for 15 minutes, then add 150 µL of chromogenic agent immediately, mix fully.
4. Measure the OD values of each well at 520 nm with microplate reader.

9. Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #1) from all standard readings. This is the absolute OD value.

3. Plot the standard curve by using absolute 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).

The sample:

1. Serum (plasma) sample:

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

ALP activity (King unit/100 mL) = (ΔA-b) ÷ a × V_1 × f

2. Tissue and cells sample:

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

ALP activity (King unit/g_{prot}) = (ΔA - b) ÷ a ÷ C_{pr} × f

[Note]

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, g_{prot}/mL

10. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (King unit/100 mL)	1.40	24.60	41.50
%CV	5.4	4.9	5.0

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (King unit/100 mL)	1.40	24.60	41.50
%CV	8.7	8.0	8.8

Recovery

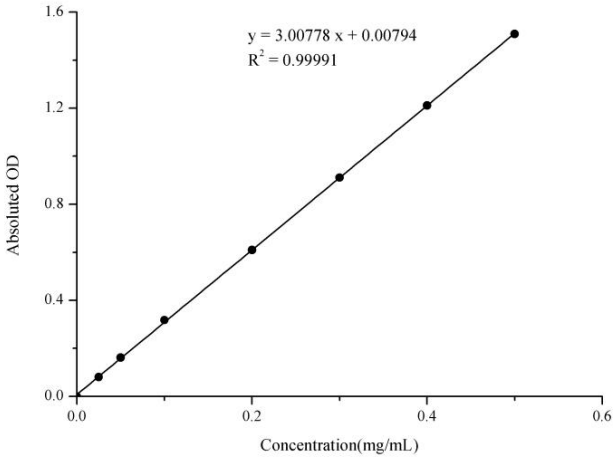
	Standard 1	Standard 2	Standard 3
Expected Conc. (mg/mL)	0.036	0.17	0.38
Observed Conc. (mg/mL)	0.034	0.16	0.36
Recovery rate (%)	95	93	94

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 O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Standard curve

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), the standard curve and data are provided below for reference only:



Standard curve data

Concentration (mg/mL)	0	0.025	0.05	0.1	0.2	0.3	0.4	0.5
Average OD	0.054	0.135	0.215	0.371	0.664	0.965	1.265	1.563
Absolute OD	0	0.081	0.161	0.317	0.610	0.911	1.211	1.509

11. Appendix II Example Analysis

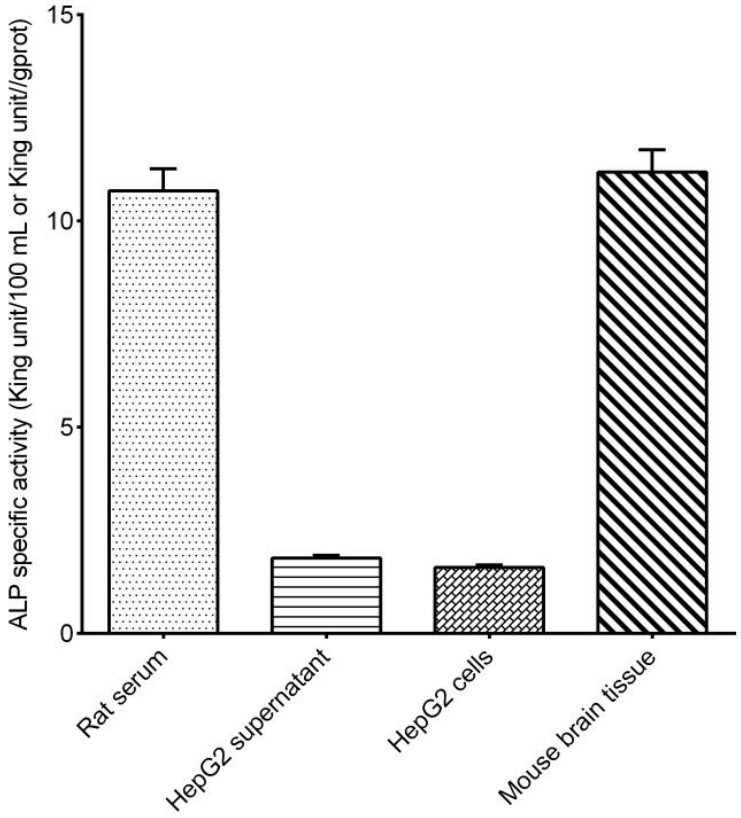
Example analysis:

Take 5 μL of rat serum and carry out the assay according to the operating steps. 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 9.23 g_{prot}/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

Detect rat serum, 10% mouse brain tissue homogenate (the concentration of protein is 0.004 g_{prot}/L diluted 2-fold), culture supernatant of HepG2 cells and HepG2 cells (the concentration of protein is 0.01 g_{prot}/mL) according to the protocol. The results are as follows:



12. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.

2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.

3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.

4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.

5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.

6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use

of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

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.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.