TECHNICAL MANUAL

Acid Phosphatase (ACP) Activity Assay Kit

- **SKU CODE:** MAES0024
- **SIZE:** 96 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Sample preparation
- Add standards and samples
- Incubate at 37°C for 10 min
- Add chromogenic agent
- Measure OD at 405 nm

2. Intended use

This kit can be used to measure acid phosphatase (ACP) activity in serum, plasma, and tissue samples.

3. Detection principle

Disodium p-nitrobenzene phosphate (p-NPP), a widely used phosphatase chromogenic substrate, forms p-nitrophenol under the action of acid phosphatase. Under alkaline conditions, p-nitrophenol is yellow and has a maximum absorption peak at 405 nm. The darker the yellow product, the higher the acid phosphatase (ACP) activity. Therefore, the activity of ACP can be calculated by measuring the OD value at 405 nm.

4. Kit components & storage

Item	Component	Size 1 (96 T)	Size 2 (500 Assays)	Storage
Reagent 1	Buffer Solution	20 mL × 1 vial	50 mL × 2 vials	-20°C, 12 months
Reagent 2	Substrate	Powder × 3 vials	Powder × 15 vials	-20°C, 12 months, protect from light
Reagent 3	Standard	Powder × 1 vial	Powder × 5 vials	-20°C, 12 months, protect from light
Reagent 4	Chromogenic Agent	24 mL × 1 vial	60 mL × 2 vials	-20°C, 12 months
	Microplate	96 wells		No requirement
	Plate Sealer	2 pieces		

5. Materials prepared by users

Instruments:

Micropipettor, Microplate reader (400-415 nm, optimum wavelength: 405 nm), 37°C Incubator

Reagents:

Double distilled water, PBS (0.01 M, pH 7.4)

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of substrate working solution:** Dissolve one vial of substrate with 1.6 mL of buffer solution. Store at -20°C for 24 hours protected from light.
3. **Preparation of 10 mmol/L standard stock solution:** Dissolve one vial of standard with 5 mL of double distilled water, mix well. Store aliquots at -20°C for 7 days protected from light.
4. **Preparation of 0.5 mmol/L standard:** Dilute 36 µL of standard stock solution with 684 µL of buffer solution. Mix well to dissolve. The 0.5 mmol/L standard should be prepared fresh.
5. **Preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 0.5 mmol/L standard solution with buffer solution to serial concentrations. The recommended dilution gradient is as follows: 0, 0.05, 0.1, 0.2, 0.25, 0.3, 0.4, 0.5 mmol/L.

7. Standard dilution table

Item	1	2	3	4	5	6	7	8
Concentration (mmol/L)	0	0.05	0.1	0.2	0.25	0.3	0.4	0.5
0.5 mmol/L standard (µL)	0	20	40	80	100	120	160	200
Buffer solution (µL)	200	180	160	120	100	80	40	0

8. 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 one 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 PBS (0.01 M, pH 7.4) with a dounce homogenizer at 4°C.

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

5. Meanwhile, determine the protein concentration of supernatant (MAES0177).

9. Sample dilution recommendations

Sample type	Dilution factor
10% Epipremnum aureum tissue homogenate	5-10
Mouse plasma	5-10
Rat plasma	5-10
Human urine	1
Human plasma	5-10
10% Rat spleen tissue homogenate	20-30
10% Rat liver tissue homogenate	20-30
10% Rat kidney tissue homogenate	20-30

10. The key points of the assay

1. Substrate working solution and standard should be stored protected from light.

2. Substrate working solution should be used within 1 day.

11. Operating steps

1. Standard well: Add 40 µL of standards with different concentrations into the standard wells. Sample well: Add 40 µL of sample into the sample wells. Control well: Add 40 µL of sample into the control wells.

2. Add 40 µL of buffer solution to the standard wells and control wells.

3. Add 40 µL of substrate working solution to the sample wells.

4. Mix thoroughly for 3 s with microplate reader and incubate at 37°C for 10 min.

5. Add 160 µL of chromogenic agent to each well.

6. Mix thoroughly for 3 s with microplate reader. Measure the OD values of each well at 405 nm with microplate reader.

12. 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 corresponding 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 µmol p-nitrophenol produced by 1 L serum (plasma) per minute hydrolysis PNPP at 37°C is defined as 1 activity unit.

ACP activity (U/L) = (ΔA - b) ÷ a ÷ T × f × 1000*

2. Tissue sample:

Definition: The amount of 1 µmol p-nitrophenol produced by 1 g tissue protein per minute hydrolysis PNPP at 37°C is defined as 1 activity unit.

ACP activity (U/gprot) = (ΔA - b) ÷ a ÷ T × f × 1000* ÷ C_{pr}

[Note]

ΔA_405: Absolute OD value, OD_{Sample} – $OD_{Control}$

f: Dilution factor of sample before test

T: Reaction time, 10 min

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

1000*: 1 mmol = 1000 µmol

13. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Intra-assay Precision Results

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	0.50	15.00	38.00
%CV	3.2	2.6	2.6

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Inter-assay Precision Results

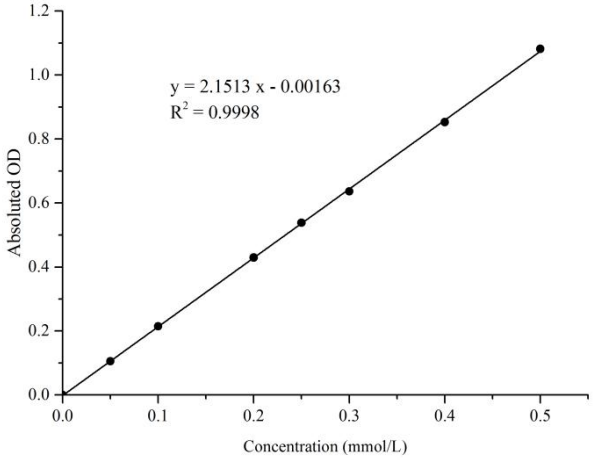
Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	0.50	15.00	38.00
%CV	4.3	4.7	4.5

Sensitivity

The analytical sensitivity of the assay is 0.2 U/L ACP. 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 data

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 reference data

Concentration (mmol/L)	0	0.05	0.1	0.2	0.25	0.3	0.4	0.5
Average OD	0.039	0.145	0.214	0.429	0.577	0.675	0.892	1.121
Absolute OD	0	0.105	0.214	0.429	0.538	0.626	0.852	1.081

14. Appendix II Example Analysis

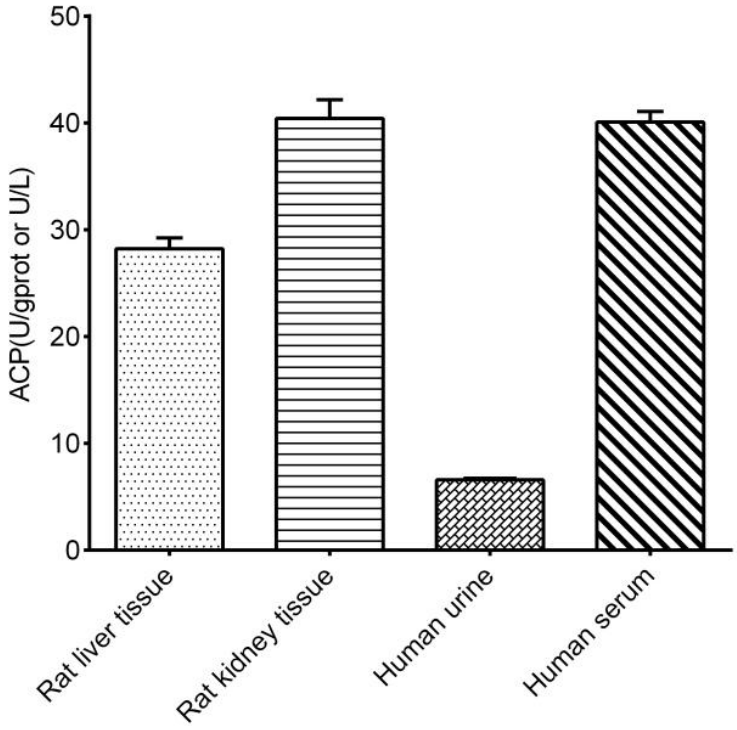
Example analysis:

For rat kidney tissue, take 10% rat kidney tissue homogenate diluted 20 times and carry out the assay according to the operation steps. The results are as follows:

Standard curve: y = 2.1298x + 0.0025, the average OD value of the sample is 0.470, the average OD value of the control is 0.06, the concentration of protein in sample is 9.47 gprot/L, and the calculation result is:

ACP activity (U/gprot) = (0.470 - 0.06 - 0.0025) ÷ 2.1298 ÷ 10 ÷ 9.47 × 20 × 1000 = 40.4 U/gprot

Detect 10% rat liver tissue homogenate (the concentration of protein is 12.22 gprot/L diluted 20 times), 10% rat kidney tissue homogenate (the concentration of protein is 9.47 gprot/L diluted 20 times), human urine and human serum (diluted 10 times) according to the protocol. The results are as follows:



15. Statement

1. This assay kit is for Research Use Only. Assay Genie will not be responsible for any arising problems or legal responsibilities caused by using the kit for clinical diagnosis or other purposes.
2. Please read the instructions carefully and calibrate the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an additional dilution or concentration should be performed on the sample.
5. It is recommended to perform a pre-test if your sample is not listed in the instruction manual.
6. The experimental results are closely related to the condition of reagents, operations, environment and other factors. Assay Genie will guarantee the quality of the kits only, and will NOT be responsible for the sample consumption caused

by using the assay kits. It is advisable to calculate the expected sample usage and reserve sufficient samples before use.

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.