TECHNICAL MANUAL

Acid Phosphatase (ACP) Activity Assay Kit

- **SKU CODE:** MAES0082
- **SIZE:** 96 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

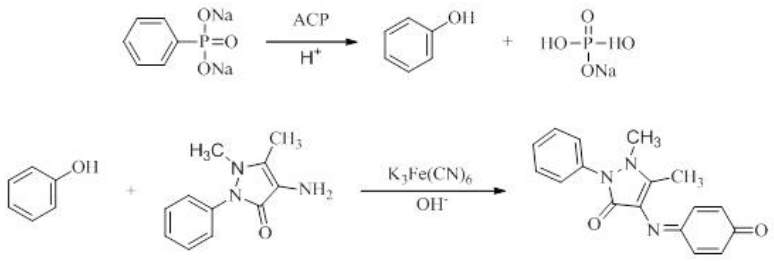
- Reagent preparation
- Sample preparation
- Add standards and samples
- Incubate and add reagents
- Measure OD values

2. Intended use

This kit can be used to measure acid phosphatase (ACP) activity in serum (plasma), pleural effusion, urine, cells, cell culture supernatant, and animal tissue samples.

3. Detection principle

Acid phosphatase decomposes disodium phenyl phosphate under acidic conditions to produce free phenol and phosphoric acid. Phenol reacts with 4-aminoantipyrine in alkaline solution and is oxidized to a red quinone derivative by potassium ferricyanide. The activity of ACP can be calculated by measuring the OD value at 520 nm.



4. Kit components & storage

Item	Component	Size 1 (96 T)	Size 2 (500 Assays)	Storage
Reagent 1	Buffer Solution	2 mL × 1 vial	10 mL × 1 vial	2-8 °C, 12 months
Reagent 2	Substrate Solution	2 mL × 1 vial	10 mL × 1 vial	2-8 °C, 12 months, protect from light
Reagent 3	Alkali Reagent	6 mL × 2 vials	60 mL × 1 vial	2-8 °C, 12 months, protect from light
Reagent 4	Chromogenic Agent	9 mL × 2 vials	45 mL × 2 vials	2-8 °C, 12 months, protect from light

Item	Component	Size 1 (96 T)	Size 2 (500 Assays)	Storage
Reagent 5	1.0 mg/mL Phenol Standard	2 mL × 1 vial	10 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 (510-530 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, add 12.5 µL of Buffer Solution and 12.5 µL of Substrate Solution, mix well. The working solution should be prepared fresh. Store at 2-8 °C for up to 1 day.
3. **Preparation of standard curve::** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 1 mg/mL phenol standard with double distilled water to serial concentrations. The recommended dilution gradient is as follows: 0, 0.1, 0.2, 0.4, 0.5, 0.6, 0.8, 1 mg/mL.

7. Sample preparation

Sample preparation:

Serum and plasma: Detect directly. If not detected on the same day, 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 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 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).

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 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 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).

Dilution of sample:

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

- Human serum: 1
- Human saliva: 1
- Chicken serum: 1
- Human urine: 1
- 10% Rat liver tissue homogenate: 4-10
- 10% Rat kidney tissue homogenate: 4-10
- HepG2 cells: 1

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4). For dilution of other sample types, please perform a pre-test 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 25 µL of working solution and mix thoroughly for 10 s with microplate reader.
3. Incubate at 37 °C for 30 min, then add 100 µL of Alkali Reagent and 150 µL of Chromogenic Agent, mix thoroughly for 10 s with microplate reader.
4. Stand for 5 min at room temperature and 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 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 mg phenol produced by 100 mL sample reacting with the substrate in 30 min is defined as 1 unit.

ACP activity (U/100 mL) = (ΔA - b) ÷ a × V × f

2. Tissue and cell sample:

Definition: The amount of 1 mg phenol produced by 1 g tissue protein reacting with the substrate in 30 min is defined as 1 unit.

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

[Note]

ΔA: Absolute OD (OD_{Sample} – OD_{Blank}).

V: The volume of sample in definition, 100 mL.

f: Dilution factor of sample before test.

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

10. 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

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/100 mL)	3.70	26.80	32.50
%CV	2.5	2.0	1.8

Inter-assay Precision

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

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/100 mL)	3.70	26.80	32.50
%CV	9.4	9.2	9.6

Recovery

Three samples of high, middle, and low concentration were tested with each concentration run 6 times in parallel to obtain an average recovery rate of 104%.

Recovery

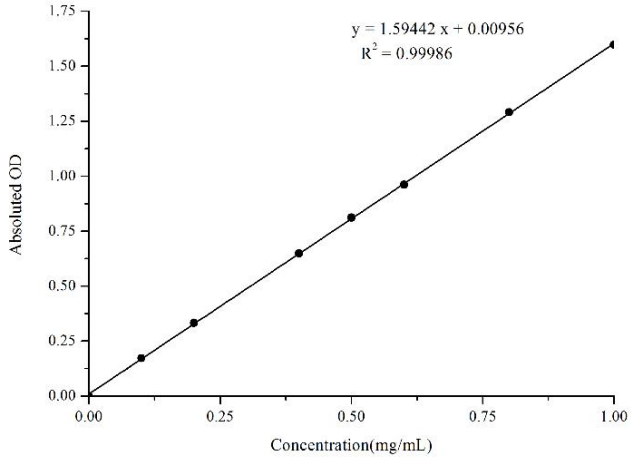
Standard 1	Standard 2	Standard 3
Expected Conc. (mg/mL): 0.15	Expected Conc. (mg/mL): 0.45	Expected Conc. (mg/mL): 0.77
Observed Conc. (mg/mL): 0.2	Observed Conc. (mg/mL): 0.5	Observed Conc. (mg/mL): 0.8
Recovery rate (%): 101	Recovery rate (%): 106	Recovery rate (%): 105

Sensitivity

The analytical sensitivity of the assay is 1.40 U/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	0.1	0.2	0.4	0.5	0.6	0.8	1
Average OD	0.061	0.234	0.394	0.710	0.873	1.022	1.352	1.659
Absolute OD	0	0.173	0.333	0.649	0.812	0.961	1.291	1.598

11. Appendix II Example Analysis

Example analysis:

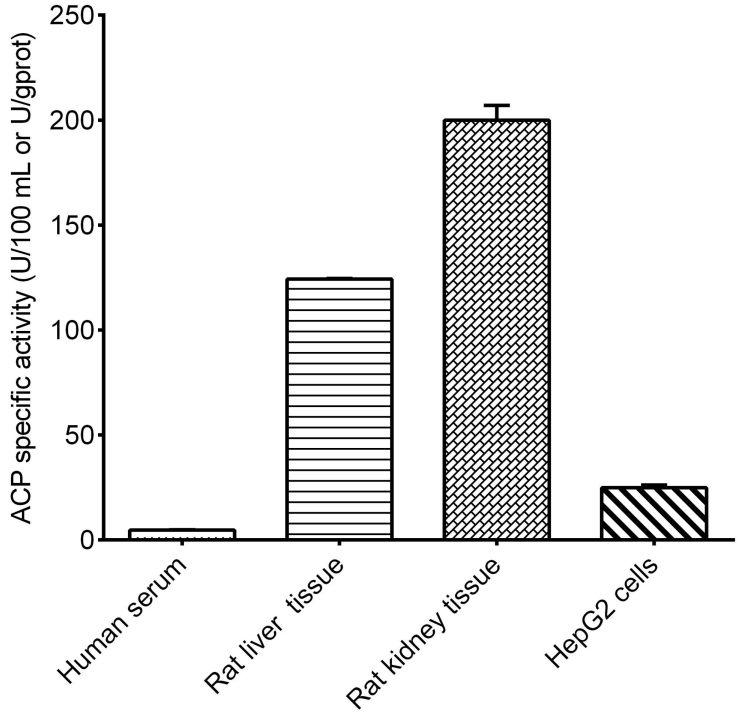
Take 5 μL of human serum and perform the assay according to the operating steps. The results are as follows:

Standard curve: y = 1.59442x + 0.00956, the average OD value of the sample is 0.153, the average OD value of the blank is 0.071, and the calculation result is:

ACP (U/100 mL) = (0.082 - 0.00956) ÷ 1.59442 × 100 = 4.54 U/100 mL

Detect human serum, 10% rat liver tissue homogenate (protein concentration is 0.013 gprot/mL, diluted 10 times), 10% rat kidney tissue homogenate (protein concentration is

0.007 gprot/mL, diluted 10 times) and HepG2 cells (protein concentration is 0.010 gprot/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. 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.