Technical Manual

Acid Phosphatase (ACP) Activity Assay Kit

- Catalogue Code: MAES0083
- Size: 100 Assays
- Research Use Only

1. Key features and Sample Types

Detection method:

Colorimetric method

Specification:

100 Assays

Range:

0.27-40 U/100 mL

Sensitivity:

0.27 U/100 mL

Storage:

2-8°C for 3 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 3 months.

Do not use components from different batches of kit.

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

2. Background

Acid phosphatase (ACP) is a kind of enzyme which catalyzes the hydrolysis of phosphate monoester to phosphoric acid under acidic conditions. There are different acid phosphatase isozymes in different organs. These isozymes differ greatly in tissue and chromosome origin, molecular weight, amino acid homology, sequence length, and resistance to L-tartrate or fluoride.

3. Intended Use

This kit can be used for detection of ACP activity in serum, plasma, urine, tissue and cells sample.

4. Detection Principle

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

5. Kit components & storage

Item	Specification	Storage
Buffer Solution	60 mL × 1 vial	2-8°C, 3 months
Substrate Solution	60 mL × 1 vial	2-8°C, 3 months, shading light
Alkali Reagent	60 mL × 2 vials	2-8°C, 3 months, shading light
Chromogenic Agent	60 mL × 3 vials	2-8°C, 3 months, shading light
Standard (1 mg/mL)	1 mL × 1 vial	2-8°C, 3 months, shading light
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Spectrophotometer (520 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:

Add alkali reagent and chromogenic agent immediately after incubation at 37°C for 30 min.

7. Reagent preparation:

1. Bring all reagents to room temperature before use.

2. Preparation of **phenol standard application solution (0.1 mg/mL):** Dilute standard (1 mg/mL) with double distilled water at a ratio of 1:9 and mix fully. Prepared the fresh solution before use.

8. Sample Preparation

Sample requirements: The sample should not contain oxalate and fluoride.

1. Serum sample:
Fresh blood should be incubated at 25°C for 30 min to clot the blood. Centrifuge the sample 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:
Place the fresh blood sample into a tube of anticoagulant and centrifuge at 700-1000g 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): PBS (0.01 M, pH 7.4) or 0.9% NaCl (μL) =1: 200. 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 PBS (0.01 M, pH 7.4) or 0.9% NaCl (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 (0.27-40 U/100 mL).

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

Sample Type:	Dilution Factor
Human milk	1
Human saliva	1
Human urine	1
Human serum	1
HepG2 cells homogenization	2-8
10% Mouse kidney tissue homogenization	8-12
10% Mouse liver tissue homogenization	8-12
10% Mouse spleen tissue homogenization	8-12

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

10. Operation Steps

1. **Blank tube:** add 50 μL of double distilled water to the corresponding wells.
   Standard tube: add 50 μL of 0.1 mg/mL Phenol standard application solution to the corresponding wells.
   Sample tube: add 50 μL of sample to the corresponding wells.

2. Successively add 500 μL of buffer solution and 500 μL of substrate solution respectively and oscillate fully with the vortex mixer.

3. Incubate at 37°C for 30 min, then add 1000 μL of alkali reagent and 1500 μL of chromogenic agent immediately, oscillate fully with the vortex mixer and stand at room temperature for 10 min.

4. Set the spectrophotometer to zero with double distilled water and measure the OD values of each tube at 520 nm with 1 cm optical path quartz cuvette.

Operation Table

	Blank tube	Standard tube	Sample tube
Double distilled water (μL)	50		
0.1 mg/mL Phenol standard application solution (μL)		50	
Sample (μL)			50
Buffer solution (μL)	500	500	500
Substrate solution (μL)	500	500	500
Mix fully and incubate at 37°C for 15 min.			
Alkali reagent (μL)	1000	1000	1000
Chromogenic agent (μL)	1500	1500	1500
Mix fully immediately, then set the spectrophotometer to zero with double distilled water and measure the OD values of each tube at 520 nm with 0.5 cm optical path quartz cuvette.			

11. Calculations

1. Serum (plasma) and other liquid sample:

Definition:100 mL of sample reacts with the substrate at 37°C for 30 min to produce 1 mg of phenol that is defined as 1 unit.

$$\text{ACP activity (U/100 mL)} = \frac{\Delta A_1}{\Delta A_2} \times m \times \frac{V_1}{V} \times f$$

2. Tissue and cell sample:

Definition:1 g of sample protein reacts with the substrate at 37°C for 30 min to produce 1 mg of phenol that is defined as 1 unit.

$$\text{ACP activity (U/gprot)} = \frac{\Delta A_1}{\Delta A_2} \times m \div [C_{pr} \times V] \times f$$

ΔA_1: OD_{sample}-OD_{blank}

ΔA_2: $OD_{standard}$-OD_{blank}

m: Phenol content of standard tube, 0.005 mg

C_{pr}: Protein concentration of tested sample, gprot/mL

V: The volume of sample, 0.05 mL

V_1: The volume of sample in definition, 100 mL

f: Dilution factor of sample before test.

12. Performance Characteristics

Detection Range	0.27-40 U/100 mL
Sensitivity	0.27 U/100 mL
Average recovery rate (%)	100
Average inter-assay CV (%)	7.1
Average intra-assay CV (%)	2.8

Analysis

Take 50 µL of rat serum, carry the assay according to the operation table.

The results are as follows:

The average OD value of the sample is 0.200, the average OD value of the blank is 0.037, the average OD value of the standard is 0.215, and the calculation result is:

$$\textbf{ACP activity (U/100 mL)} = \frac{0.200-0.037}{0.215-0.037} \times 0.005 \times \frac{100}{0.05} = 9.16 \text{ (U/100 mL)}$$

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.

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