Technical Manual

Acid Phosphatase (ACP) Activity Assay Kit

- **Catalogue Code: MAES0024**
- **Size: 96T**
- **Research Use Only**

1. Key Features and Sample Types

Detection method:

Colorimetric method

Specification:

96T

Range:

0.2–50 U/L

Sensitivity:

0.2 U/L

Storage:

-20°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.

2. Background

Acid phosphatase (ACP) is a kind of acidic hydrolytic enzyme with high content in lysosomes which catalyzes the hydrolysis of phosphate monoester to phosphoric acid under acidic conditions. ACP is ubiquitous in nature, from low organisms such as escherichia coli and yeast to higher animal and plant tissues, as well as body fluids, human liver, and prostate.

3. Intended Use

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

4. Detection Principle

Disodium p-nitrobenzene phosphate (PNPP), a widely used phosphatase chromogenic substrate, can form 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 is, the higher of the ACP activity is. Therefore, the activity of ACP can be calculated by measuring the OD value at 405 nm.

5. Kit Components & Storage

Item	Specification	Storage
Buffer Solution	20 mL × 1 vial	-20°C, 3 months
Substrate	Lyophilized × 3 vials	-20°C 3 months, avoid direct sunlight
Standard	Lyophilized × 1 vial	-20°C 3 months, avoid direct sunlight
Chromogenic Agent	24 mL × 1 vial	-20°C, 3 months
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Vortex mixer
- Water bath
- Microplate Reader (400-415 nm, optimum wavelength: 405 nm)
- Tips (10 µL, 200 µL, 1000 µL)
- EP tubes (1.5 mL, 2 mL)
- PBS (0.01 M, pH 7.4)
- Double distilled water

6. Assay Notes:

1. Substrate working solution and standard should be stored away from direct sunlight.

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

7. Reagent Preparation:

1. Bring all reagents to room temperature before use.

2. Preparation of **substrate working solution:** Dissolve substrate with 1.6 mL of buffer solution. The prepared solution can be stored at -20°C away from direct sunlight for 24 hours.

3. Preparation of **10 mmol/L standard solution:** Dissolve a vial of standard power with 5 mL double distilled water and mix fully. The prepared solution can be aliquoted into smaller quantities and stored at -20°C for 7 days away from direct sunlight.

4. Preparation of **0.5 mmol/L standard solution:** Mix the 10 mmol/L standard solution and buffer solution at the ratio of 1: 9. Prepare the needed amount before use and store it away from direct sunlight before detection.

8. Sample Preparation

1. Serum/plasma sample:
Detect the sample directly.

2. Tissue sample:
Weigh the tissue accurately. Add PBS (0.01 M, pH 7.4) in a weight (g): volume (mL) ratio of 1: 9, homogenize mechanically in ice water bath to break cells fully. Then centrifuge at 10000 g for 10 min at 4°C and collect the supernatant for measurement. Meanwhile, determine the protein concentration of supernatant (MAES0177).

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 (0.2–50 U/L).

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

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

Note: The diluent is PBS (0.01 M, pH 7.4).

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 405 nm

Plate Set Up:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S1′	S9′	S17′	S25′	S33′
B	B	B	S2	S10	S18	S26	S34	S2′	S10′	S18′	S26′	S34′
C	C	C	S3	S11	S19	S27	S35	S3′	S11′	S19′	S27′	S35′
D	D	D	S4	S12	S20	S28	S36	S4′	S12′	S20′	S28′	S36′
E	E	E	S5	S13	S21	S29	S37	S5′	S13′	S21′	S29′	S37′
F	F	F	S6	S14	S22	S30	S38	S6′	S14′	S22′	S30′	S38′
G	G	G	S7	S15	S23	S31	S39	S7′	S15′	S23′	S31′	S39′
H	H	H	S8	S16	S24	S32	S40	S8′	S16′	S24′	S32′	S40′

Note: A-H, standard wells; S1-S40, sample wells; S1′-S40′, control wells.

10. Operation Steps

The preparation of standard curve

Dilute 0.5 mmol/L standard solution with buffer solution to a serial concentration. The recommended dilution gradient is as follows: 0, 0.05, 0.1, 0.2, 0.25, 0.3, 0.4, 0.5 mmol/L.

The measurement of samples

1. **Standard well:** Take 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 into the standard wells and control wells.

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

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

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

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

Operation Table

	Standard well	Sample well	Control well
Standards with different concentrations (μL)	40		
Sample (μL)		40	40
Buffer solution (μL)	40		40
Substrate working solution (μL)		40	
Mix fully with microplate reader for 3 s and incubate at 37°C for 10 min.			
Chromogenic agent (μL)	160	160	160
Mix fully with microplate reader for 3 s. Measure the OD values of each well at 405 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 μmol p-nitrophenol produced by 1L serum (plasma) per minute hydrolysis PNPP at 37°C defined as 1 activity unit.

$$\text{ACP activity (U/L)} = (\Delta A - b) \div a \div T \times f \times 1000^*$$

2. Tissue sample:

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

$$\text{ACP activity (U/L)} = (\Delta A - b) \div a \div T \times f \times 1000^* \div C_p$$

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} – $OD_{Control}$)

f: Dilution factor of sample before test

T: Reaction time, 10 min

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

1000*: 1 mmol=1000 μmoL

12. Performance Characteristics

Detection Range	0.2–50 U/L
Sensitivity	0.2 U/L
Average inter-assay CV (%)	4.5
Average intra-assay CV (%)	2.8

Analysis

For rat kidney tissue, take 10% rat kidney tissue homogenate diluted 20 times, and carryout the assay according to the operation table.

The results are as follows:

Standard curve: y = 2.1298 x+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:

$$\text{ACP activity (U/gprot)} = \mathbf{(0.470\text{-}0.06\text{-}0.0025) \div 2.1298 \div 10 \div 9.47 \times 20 \times 1000}$$

$$= \mathbf{(40.4 U/gprot)}$$

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

Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent 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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