Technical Manual

Tartrate Resistant Acid Phosphatase (TRAP) Activity Assay Kit

- **Catalogue Code: MAES0271**
- **Size: 96T**
- **Research Use Only**

1. Key features and Sample Types

Detection method:
Colorimetric method

Specification:
96T

Range:
9.22-100 U/L

Sensitivity:
9.22 U/L

Storage:
-20°C for 12 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 12 months.

Do not use components from different batches of kit.

2. Intended Use

This kit can measure tartrate resistant acid phosphatase (TRAP) activity in serum, plasma, urine and animal tissue samples.

3. Detection Principle

The chromogenic agent can be catalyzed by acid phosphatase to produce p-nitrophenol in acidic condition. P-nitrophenol has a maximum absorption at 405 nm. The activity of TRAP can be calculated by measuring the amount of produced p-nitrophenol. The activity of acid phosphatase being detected in the presence of tartric acid is regarded as TRAP activity.

4. Kit components & storage

Item	Specification	Storage
Buffer Solution	24 mL × 1 vial	-20°C, 12 months
Substrate	Powder × 2 vials	-20°C, 12 months, shading light
Tartaric Acid Solution	1.4 mL × 2 vials	-20°C, 12 months
Standard Substance	Powder × 2 vials	-20°C, 12 months, shading light
Chromogenic Agent	15 mL × 1 vial	-20°C, 12 months
Microplate	96 wells	
Plate Sealer	2 pieces	

Materials required but not supplied

- Microplate reader (410-415 nm, optimum wavelength: 405 nm)
- Incubator (37°C)
- Normal saline (0.9% NaCl)

5. Assay Notes:

Substrate working solution and the reaction working solution are easily decomposed in light. Avoid light during use.

6. Reagent Preparation

1. Equilibrate all reagents to room temperature (25°C) before use.

2. Preparation of substrate working solution:
   Dissolve one vial of substrate with 0.5 mL of double distilled water, mix well. Keep substrate working solution on ice during use. Store at -20°C for 2 days protected from light.

3. Preparation of reaction working solution:
   Before testing, please prepare sufficient reaction working solution according to the test wells. For example, prepare 126 μL of reaction working solution (mix well 119 μL of buffer solution and 7 μL of substrate working solution). Keep reaction working solution on ice during use. The reaction working solution should be used up within 6 hours.

4. Preparation of 10 mmol/L standard solution:
   Dissolve one vial of standard substance with 1 mL of double distilled water, mix well. Store at -20°C for 2 days protected from light.

5. Preparation of 1 mmol/L standard solution:
   Dilute 10 μL of 10 mmol/L standard solution with 900 μL of buffer solution, mix well. The 1 mmol/L standard solution should be prepared on spot. Keep 1 mmol/L standard solution on ice protected from light during use.

6. Preparation of standard curve:
   Always prepare a fresh set of standards. Discard working standard dilutions after use.

   Dilute 1 mmol/L standard solution with buffer solution to a serial concentration. The recommended dilution gradient is as follows: 0, 0.2, 0.3, 0.4, 0.6, 0.8, 0.9, 1.0 mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**0.2**	**0.3**	**0.4**	**0.6**	**0.8**	**0.9**	**1.0**
1 mmol/L standard (μL)	0	40	80	100	120	140	160	200
Buffer solution (μL)	200	160	140	120	80	40	20	0

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7. Sample Preparation

Serum, plasma, and urine:

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) with a dounce homogenizer at 4°C.
4. Centrifuge at 10000×g for 10 min 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 200 µL normal saline (0.9% NaCl) with a ultrasonic cell disruptor at 4°C.
4. Centrifuge at 10000×g for 10 min 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):

Sample Type	Dilution Factor
Goat plasma	1
Human urine	1
Porcine serum	1
Rat plasma	1-2
10% Mouse liver tissue homogenate	5-8
10% Mouse kidney tissue homogenate	3-5
10% Mouse lung tissue homogenate	2-5
10% Mouse brain tissue homogenate	3-5
1×10^6 HL-60 cells	1
1×10^6 293T cells	1

Note: The diluent is normal saline (0.9% NaCl). For the dilution of other sample types, please do pretest to confirm the dilution factor.

8. Operation Steps

1. Standard well: Add 20 μL of standard solution with different concentrations into the corresponding wells.
   Sample well: Add 20 μL of sample into sample well.
   Control well: Add 20 μL of sample into control well.
2. Add 120 μL of reaction working solution into standard well and sample well.
   Add 120 μL of buffer solution into control well.
3. Add 20 μL of tartaric acid solution into each well.
4. Mix fully with microplate reader for 3 s, incubate at 37°C for 10 min.
5. Add 100 μL of chromogenic agent into each well and mix fully with microplate reader for 3 s, stand at room temperature for 2 min.
6. Measure the OD values of each well at 405 nm with microplate reader.
   Sample tube: add 50 μL of sample to the tubes.

9. Calculations

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 absoluted OD value.
3. Plot the standard curve by using absoluted 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, urine sample:**

Definition: The amount of TRAP in 1 L serum, plasma or urine sample per 1 minute that hydrolyze the substrate to produce 1 μmol p-nitrophenol at 37°C is defined as 1 unit.

$$\text{TRAP activity (U/L)} = (\Delta A_{405} - b) \div a \div T \times 1000 \times f$$

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2. **Tissue and cell sample:**
Definition: The amount of TRAP in 1 g tissue or cell protein per 1 minute that hydrolyze the substrate to produce 1 μmol p-nitrophenol at 37°C is defined as 1 unit.

$$\text{TRAP activity (U/gprot)} = (\Delta A_{405} - b) \div a \div T \times 1000 \div C_{pr} \times f$$

Note:

ΔA405: ODSample – ODcontrol.

T: The time of reaction, 10 min.

Cpr: The concentration of protein in sample, gprot/L.

1000: 1 mmol/L = 1000 μmol/L.

f: Dilution factor of sample before tested

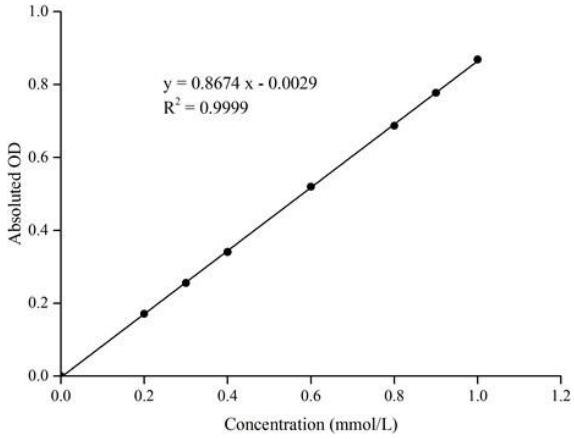
10. Performance Characteristics

Detection Range	9.22-100 U/L
Sensitivity	9.22 U/L
Average recovery rate (%)	101
Average inter-assay CV (%)	2.3
Average intra-assay CV (%)	2

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), so the standard curve and data are provided as below for reference only:

Concentration (mmol/L)	**0**	**0.2**	**0.3**	**0.4**	**0.6**	**0.8**	**0.9**	**1.0**
OD value	0.079	0.25	0.332	0.417	0.601	0.767	0.852	0.953
	0.078	0.249	0.336	0.421	0.595	0.764	0.86	0.941
Average OD	0.078	0.249	0.334	0.419	0.598	0.765	0.856	0.947
Absoluted OD	0	0.171	0.256	0.340	0.519	0.687	0.777	0.868



Analysis

Take 20 μL of 10% mouse liver tissue homogenate which dilute for 5 times in normal saline (0.9% NaCl) and carry the assay according to the operation steps. The results are as follows: Standard curve: y = 0.8674 x – 0.003, the average OD value of the control is 0.108, the average OD value of the sample is 0.334, ΔA405 = ODSample – ODcontrol = 0.334 – 0.108 = 0.226, the concentration of protein in sample is 6.77 gprot/L, and the calculation result is:

TRAP activity = (0.226 + 0.003) ÷ 0.8674 ÷ 10 × 1000 ÷ 6.77 × 5 = 19.5 U/gprot (U/gprot)

Detect 10% Mouse kidney tissue homogenate (the concentration of protein is 6.77 gprot/L, dilute for 5 times), 10% Mouse liver tissue homogenate (the concentration of protein is 11.45 gprot/L, dilute for 5 times), 10^6 HL-60 cell (the concentration of protein is 1.22 gprot/L), rat plasma (dilute for 2 times) according to the protocol.

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.

Notes:

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