TECHNICAL MANUAL

Tartrate Resistant Acid Phosphatase (TRAP) Activity Assay Kit

- **SKU CODE:** MAES0271
- **SIZE:** 96 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Intended use

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

2. Detection principle

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

3. Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	24 mL × 1 vial	-20°C, 12 months
Reagent 2	Substrate	Powder × 2 vials	-20°C, 12 months, shading light
Reagent 3	Tartaric Acid Solution	1.4 mL × 2 vials	-20°C, 12 months
Reagent 4	10 mmol/L Standard Solution	1 mL × 1 vial	-20°C, 12 months, shading light
Reagent 5	Chromogenic Agent	15 mL × 1 vial	-20°C, 12 months
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

4. Materials prepared by users

Instruments:

Microplate reader (410-415 nm, optimum wavelength: 405 nm), Incubator (37°C)

Reagents:

Normal saline (0.9% NaCl)

5. Reagent preparation

1. Equilibrate all reagents to room temperature 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, 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 within 6 hours.
4. **Preparation of 1 mmol/L standard solution:** Before testing, prepare sufficient 1 mmol/L standard solution. For example, prepare 1000 µL of 1 mmol/L standard solution (mix well 100 µL of 10 mmol/L standard solution and 900 µL of double distilled water). The 1 mmol/L standard solution should be prepared fresh and protected from light. The prepared solution should be used within 1 hour.
5. **The 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.

6. Sample preparation

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 10,000×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 (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 200 µL normal saline (0.9% NaCl) with an ultrasonic cell disruptor at 4°C.

4. Centrifuge at 10,000×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 (MAES0177).

Dilution of sample

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

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 perform a pretest to confirm the dilution factor.

7. The key points of the assay

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

8. Operating 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.

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 by using absolute 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 hydrolyzes the substrate to produce 1 µmol p-nitrophenol at 37°C is defined as 1 unit.

TRAP activity (U/L) = (ΔA405 - b) ÷ a ÷ T × 1000 × f

2. Tissue and cell sample:

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

TRAP activity (U/gprot) = (ΔA405 - b) ÷ a ÷ T × 1000 ÷ C_{pr} × f

[Note]

ΔA405: OD_{Sample} – $OD_{control}$.

T: The time of reaction, 10 min.

C_{pr}: The concentration of protein in sample, gprot/L.

1000: 1 mmol/L = 1000 µmol/L.

f: Dilution factor of sample before tested

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/L)	16.80	34.50	87.00
%CV	2.3	2.0	1.7

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/L)	16.80	34.50	87.00
%CV	2.5	2.4	2.4

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration 6 times in parallel to get the average recovery rate of 101%.

Recovery

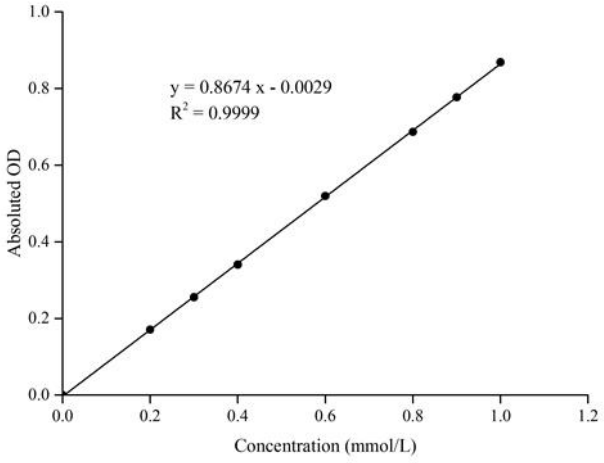
	Standard 1	Standard 2	Standard 3
Expected Conc. (mmol/L)	0.25	0.52	0.84
Observed Conc. (mmol/L)	0.3	0.5	0.9
Recovery rate (%)	101	100	102

Sensitivity

The analytical sensitivity of the assay is 0.911 U/L. 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

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

Concentration (mmol/L)	OD value		Average OD	Absolute OD
1.0	0.953	0.941	0.947	0.868

11. Appendix II Example Analysis

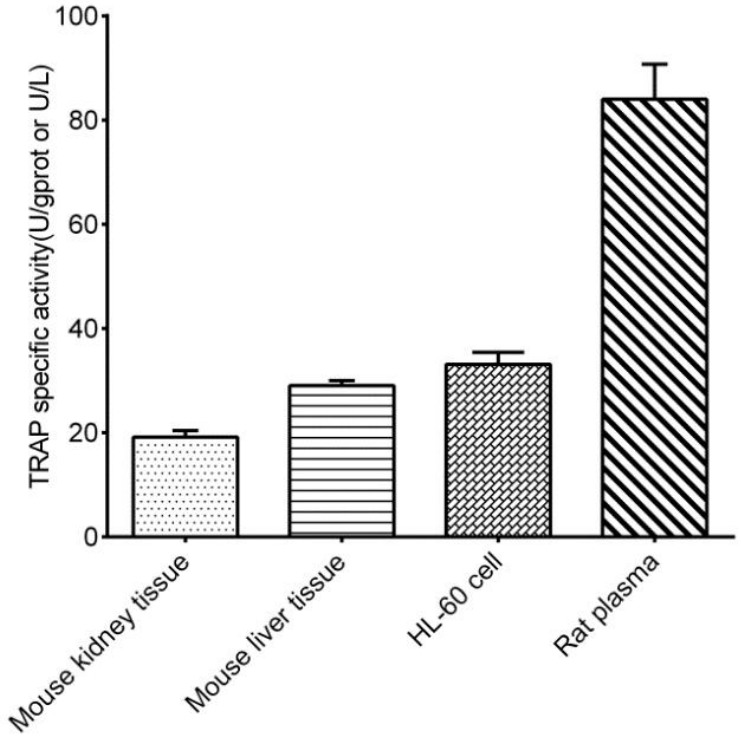
Example analysis:

Take 20 μL of 10% mouse liver tissue homogenate which is diluted 5 times in normal saline (0.9% NaCl) and carry out the assay according to the operating steps. The results are as follows:

Standard curve: y = 0.8674x – 0.003, the average OD value of the control is 0.108, the average OD value of the sample is 0.334, ΔA405 = OD_{Sample} – $OD_{control}$ = 0.334 – 0.108 = 0.226, the concentration of protein in sample is 6.77 gprot/L, and the calculation result is:

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

Detect 10% mouse kidney tissue homogenate (the concentration of protein is 6.77 gprot/L, diluted 5 times), 10% mouse liver tissue homogenate (the concentration of protein is 11.45 gprot/L, diluted 5 times), 10^6 HL-60 cells (the concentration of protein is 1.22 gprot/L), rat plasma (diluted 2 times) according to the protocol. The result is 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. Appropriate 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.