（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K871-M

Specification：96T(40 samples)

Measuring instrument：Microplate reader（410-415 nm）

Detection range：0.911-100 U/L

Elabscience®Tartrate Resistant Acid Phosphatase (TRAP) Activity Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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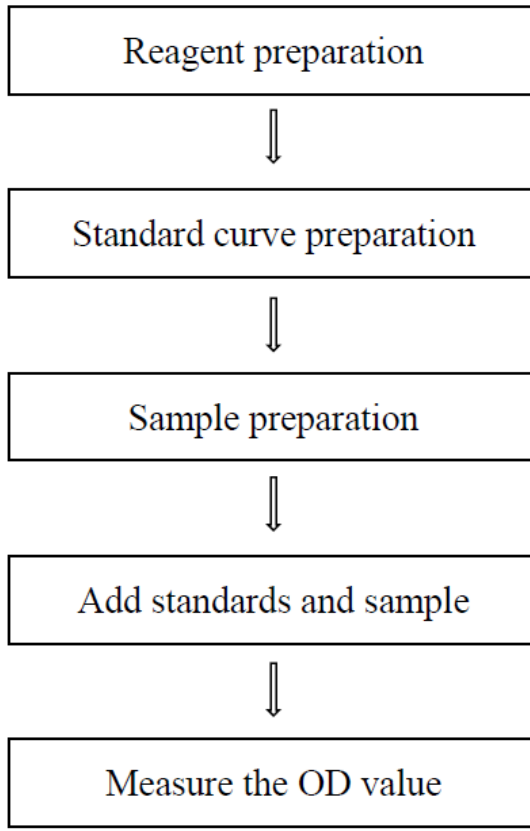
Assay summary



Intended use

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

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.

Kit components & storage

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

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

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

Reagents:

Normal saline (0.9% NaCl)

Reagent preparation

① Equilibrate all the 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 ℃ 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 ℃ 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 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. 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	60	80	120	160	180	200
Buffer solution (μL)	200	160	140	120	80	40	20	0

Sample preparation

① Sample preparation

Serum, plasma and urine: detect directly. If not detected on the same day, the serum or plasma can be stored at -80℃ 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 (E-BC-K318-M).

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 (E-BC-K318-M).

② Dilution of sample

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.

The key points of the assay

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

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 ℃ 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.

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 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℃ is defined as 1 unit.

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

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℃ 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]

ΔA_{405}: $OD_{Sample} - OD_{control}$.

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

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

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.

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 for 6 times parallelly to get the average recovery rate of 101%.

	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

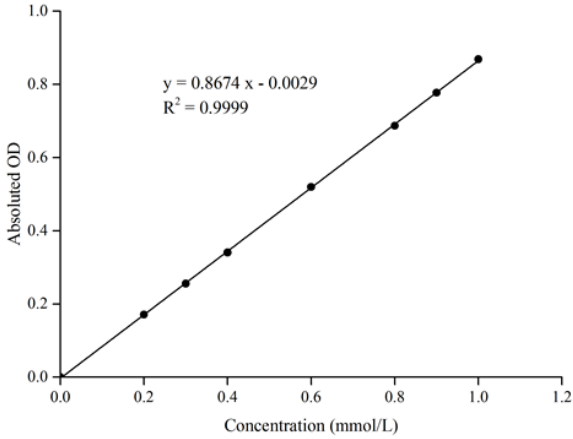
Sensitivity

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.

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



Appendix II Example Analysis

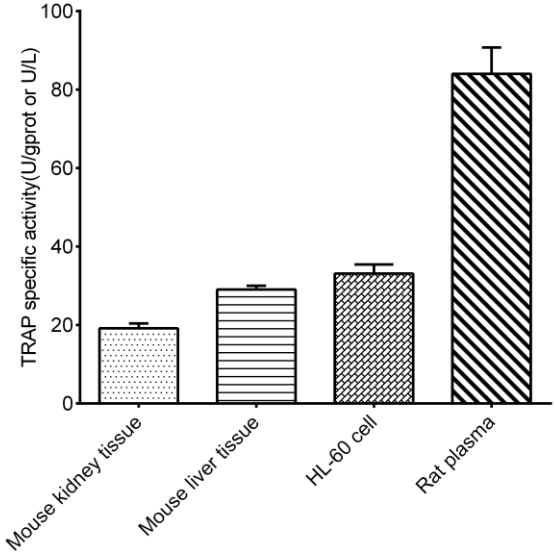
Example analysis:

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, $\Delta A_{405} = OD_{Sample} - OD_{control} = 0.334 - 0.108 = 0.226$, the concentration of protein in sample is 6.77 gprot/L, and the calculation result is:

$$\text{TRAP activity (U/gprot)} = (0.226 + 0.003) \div 0.8674 \div 10 \times 1000 \div 6.77 \times 5 = 19.5 \text{ U/gprot}$$

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, the result is as follows:



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.