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

Catalog No：E-BC-K872-M

Specification：96T(40 samples)

Measuring instrument：Microplate reader（390-415 nm）

Detection range：0.61 - 49.37U/L

Elabscience® Fluoride Resistant Acid Phosphatase (FRAP) 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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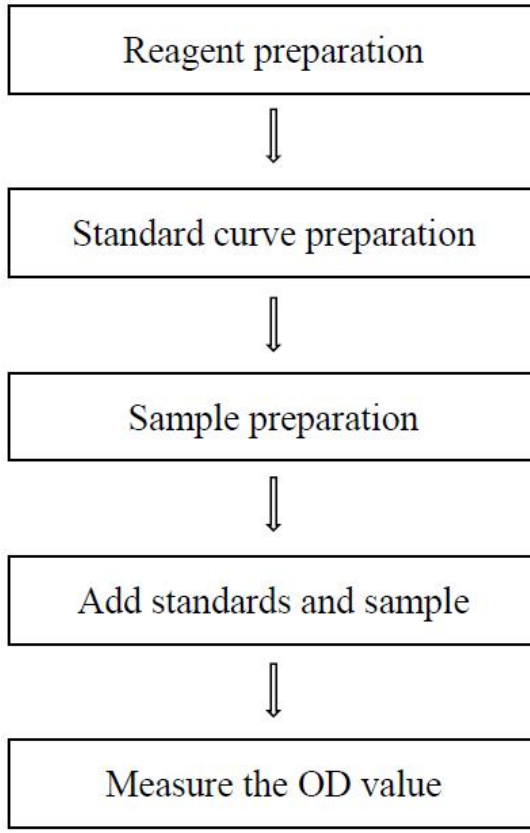
Assay summary



Intended use

This kit can measure fluoride resistant acid phosphatase(FRAP) activity in serum (plasma), cells and animal tissue samples.

Detection principle

Fluoride resistant acid phosphatase (FRAP) is not inhibited by fluoride ions. It catalyzes the substrate to produce p-nitrophenol, which has a maximum absorption peak at 405 nm. The activity of FRAP can be calculated by measuring the OD value at 405 nm.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	20 mL × 1 vial	-20°C, 12 months
Reagent 2	Fluoride Ion Solution	0.5 mL × 1 vial	-20°C, 12 months
Reagent 3	Substrate Solution	1 mL × 1 vial	-20°C, 12 months, shading light
Reagent 4	Chromogenic Agent	14 mL×1 vial	-20°C, 12 months
Reagent 5	10 mmol/L Standard Solution	1 mL × 1 vial	-20°C, 12 months, shading light
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
	Sample Layout Sheet	1 piece	

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 (390-415 nm, optimum wavelength: 405 nm), Incubator

Reagents:

Double distilled water, Normal saline (0.9% NaCl)

Reagent preparation

① Equilibrate all reagents to room temperature before use.

② Preparation of fluoride ion working solution:

Before testing, please prepare sufficient fluoride ion working solution according to the test wells. For example, prepare 400 μL of fluoride ion working solution (mix well 5 μL of fluoride ion solution and 395 μL of double distilled water). The fluoride ion working solution should be prepared on spot and used up within 1 hours.

③ Preparation of substrate working solution:

For each well, prepare 20 μL of substrate working solution (mix well 4 μL of substrate solution and 16 μL of double distilled water). The substrate working solution should be prepared on spot and used up within 1 hours.

④ Preparation of 0.5 mmol/L standard solution:

Dilute 45 μL of 10 mmol/L standard solution with 855 μL of double distilled water, mix well. Keep on ice protected from light during use. The 0.5 mmol/L standard solution should be prepared on spot and used up within 1 hours.

⑤ Preparation of measuring working solution:

For each well, prepare 80 μL of measuring working solution (mix well 60 μL of buffer solution and 20 μL of substrate working solution) The measuring working solution should be prepared on spot.

⑥ The preparation of standard curve:

Always prepare a fresh set of standards. Discard working standard dilutions

after use.

Dilute 0.5 mmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 0.1, 0.15, 0.2, 0.3, 0.35, 0.4, 0.5 mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**0.1**	**0.15**	**0.2**	**0.3**	**0.35**	**0.4**	**0.5**
0.5 mmol/L standard (μL)	0	40	60	80	120	140	160	200
Double distilled water (μL)	200	160	140	120	80	60	40	0

Sample preparation

① Sample preparation

Serum and plasma: 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 minto 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
10% Mouse liver tissue homogenate	3-7
10% Mouse kidney tissue homogenate	3-7
10% Mouse heart tissue homogenate	3-7
10% Mouse lung tissue homogenate	3-7
Rat plasma	5-7
Human serum	1-3
Bovine serum	1-3
1×10^6 HL-60 cell	1
1×10^6 293T cell	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.

Operating steps

① Standard well: Take 20 μL of standard solution with different concentrations to the corresponding wells.

Sample well: Take 20 μL of sample to the corresponding wells.

Control well: Take 20 μL of sample to the corresponding wells.

② Add 20 μL of fluoride ion working solution to each well.

③ Add 80 μL of measuring working solution to the standard wells and sample wells. Add 80 μL of buffer solution to control wells.

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

⑤ Add 100 μL of chromogenic agent to each well.

⑥ Mix fully with microplate reader and measure the OD value of each well at 405 nm.

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) and urine sample:

Definition: The amount of FRAP in 1 L serum (plasma) or urine that hydrolyze the substrate to produce 1 μmol p-nitrophenol in 1 min at 37°C is defined as 1 unit.

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

2. Tissue and cell sample:

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

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

[Note]

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

T: The time of reaction, 10 min.

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

f: Dilution factor of sample before test.

1000*: 1 mmol/L = 1000 μmol/L

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)	3.50	18.40	36.70
%CV	3.8	3.5	3.5

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)	3.50	18.40	36.70
%CV	7.6	8.2	8.2

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 102%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (mmol/L)	0.13	0.25	0.37
Observed Conc. (mmol/L)	0.1	0.3	0.4
Recovery rate (%)	101	104	101

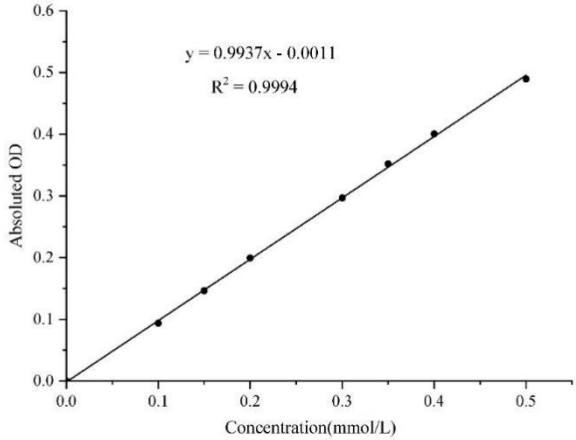
Sensitivity

The analytical sensitivity of the assay is 0.61 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**	**0.1**	**0.15**	**0.2**	**0.3**	**0.35**	**0.4**	**0.5**
Average OD	0.054	0.148	0.201	0.254	0.351	0.406	0.455	0.544
Absoluted OD	0	0.094	0.147	0.2	0.297	0.352	0.401	0.490



Appendix II Example Analysis

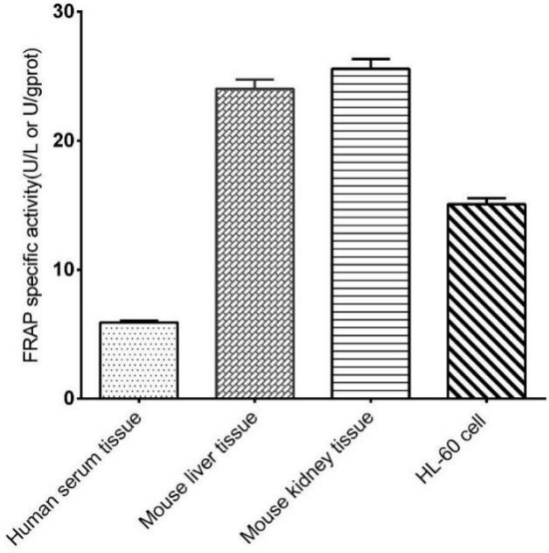
Example analysis:

For 10% rat liver tissue homogenate, dilute for 5 times, take 20 μL and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.9937x - 0.0011, the average OD value of the control is 0.078, the average OD value of the sample is 0.331, the concentration of protein in sample is 5.34 gprot/L, and the calculation result is:

$$\text{FRAP activity (U/gprot)} = (0.331 - 0.078 + 0.0011) \div 0.9937 \div 10 \times 5 \div 5.34 \times 1000 = 24.03 \text{ U/gprot}$$

Detect Human serum (dilute for 2 times), 10% mouse liver tissue homogenate (the concentration of protein is 5.34 gprot/L, dilute for 5 times), 10% mouse kidney tissue homogenate (the concentration of protein is 4.02 gprot/L, dilute for 5 times) and HL-60 cell (the concentration of protein is 0.59 gprot/L) 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.