TECHNICAL MANUAL

Fluoride Resistant Acid Phosphatase (FRAP) Activity Assay Kit

- **SKU CODE:** MAES0272
- **SIZE:** 96 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Sample preparation
- Add standards and samples
- Incubate at 37°C
- Add chromogenic agent
- Measure OD at 405 nm

2. Intended use

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

3. 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.

4. 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, protect from 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, protect from light
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

5. Materials prepared by users

Instruments:

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

Reagents:

Double distilled water, Normal saline (0.9% NaCl)

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of fluoride ion working solution:** Before testing, prepare sufficient fluoride ion working solution according to the number of test wells. For example, to prepare 400 µL of fluoride ion working solution, mix 5 µL of fluoride ion solution with 395 µL of double distilled water. The fluoride ion working solution should be prepared fresh and used within 1 hour.
3. **Preparation of substrate working solution:** For each well, prepare 20 µL of substrate working solution by mixing 4 µL of substrate solution with 16 µL of double distilled water. The substrate working solution should be prepared fresh and used within 1 hour.
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 and mix well. Keep on ice protected from light during use. The 0.5 mmol/L standard solution should be prepared fresh and used within 1 hour.
5. **Preparation of measuring working solution:** For each well, prepare 80 µL of measuring working solution by mixing 60 µL of buffer solution with 20 µL of substrate working solution. The measuring working solution should be prepared fresh.
6. **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 serial concentrations. The recommended dilution gradient is: 0, 0.1, 0.15, 0.2, 0.3, 0.35, 0.4, 0.5 mmol/L.

7. Sample preparation

Sample preparation:

Serum and plasma: Detect directly. If not detected on the same day, serum or plasma can be stored at -80°C for one 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 factors for different samples are as follows (for reference only):

- 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 dilution of other sample types, please perform a pretest to confirm the dilution factor.

8. Operating steps

1. Standard wells: Add 20 µL of standard solution at different concentrations to the corresponding wells. Sample wells: Add 20 µL of sample to the corresponding wells. Control wells: Add 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 thoroughly with microplate reader and incubate at 37°C for 10 min.
5. Add 100 µL of chromogenic agent to each well.
6. Mix thoroughly with microplate reader and measure the OD value of each well at 405 nm.

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 using absolute OD value of standard as y-axis and corresponding concentration as x-axis. 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 hydrolyzes the substrate to produce 1 µmol p-nitrophenol in 1 min at 37°C is defined as 1 unit.

FRAP activity (U/L) = (ΔA_405 - b) ÷ a ÷ T × f × 1000*

2. Tissue and cell sample:

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

FRAP activity (U/gprot) = (ΔA_405 - b) ÷ a ÷ C_{pr} ÷ T × f × 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

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)	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.

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	3.50	18.40	36.70
%CV	7.6	8.2	8.2

Recovery

Three samples of high, middle, and low concentrations were tested with 6 replicates for each concentration to obtain an average recovery rate of 102%.

Recovery

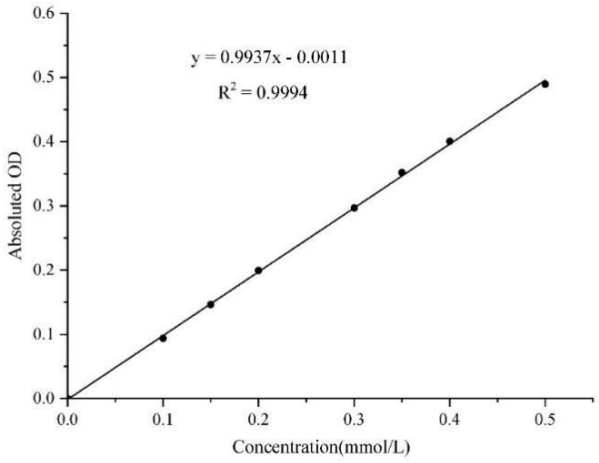
	Standard 1	Standard 2	Standard 3
Expected Conc. (mmol/L)	0.13	0.25	0.37
Observed Conc. (mmol/L)	0.1	0.3	0.4
Recovery rate (%)	101	104	101

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.

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)	Average OD	Absolute OD
0	0.054	0
0.1	0.148	0.094

Concentration (mmol/L)	Average OD	Absolute OD
0.15	0.201	0.147
0.2	0.254	0.2
0.3	0.351	0.297
0.35	0.406	0.352
0.4	0.455	0.401
0.5	0.544	0.490

11. Appendix II Example Analysis

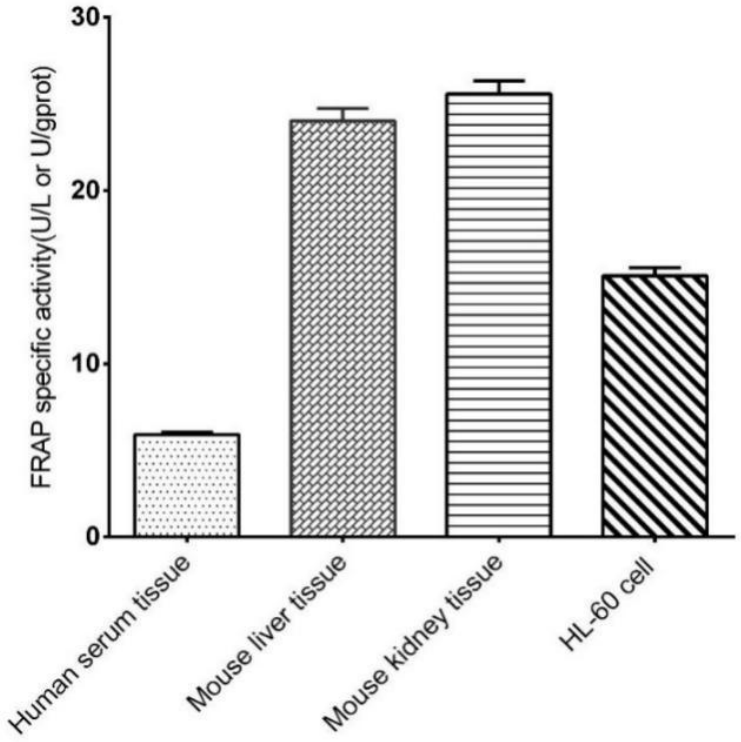
Example analysis:

For 10% rat liver tissue homogenate, dilute 5 times, take 20 µL and carry out the assay according to the operating 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:

FRAP activity (U/gprot) = (0.331 - 0.078 + 0.0011) ÷ 0.9937 ÷ 10 × 5 ÷ 5.34 × 1000 = 24.03 U/gprot

Detect human serum (dilute 2 times), 10% mouse liver tissue homogenate (protein concentration 5.34 gprot/L, dilute 5 times), 10% mouse kidney tissue homogenate (protein concentration 4.02 gprot/L, dilute 5 times) and HL-60 cell (protein concentration 0.59 gprot/L) according to the protocol. The results are 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.