



PRODUCT INFORMATION & MANUAL

Fluoride Resistant Acid Phosphatase/FRAP Activity Assay Kit (Colorimetric)

NBP3-25797

For research use only.
Not for diagnostic or therapeutic
procedures.

www.novusbio.com - P: 303.730.1950 - P: 888.506.6887 - F: 303.730.1966 - technical@novusbio.com

Novus kits are guaranteed for 6 months from date of receipt

Fluoride Resistant Acid Phosphatase/ FRAP Activity Assay Kit (Colorimetric)

Catalog No: NBP3-25797

Method: Colorimetric method

Specification: 96T (Can detect 40 samples without duplication)

Instrument: Microplate reader

Sensitivity: 0.61 U/L

Detection range: 0.61- 49.37U/L

Average intra-assay CV (%):3.6

Average inter-assay CV (%): 8.0

Average recovery rate (%): 102

- ▲ This kit is for research use only.
- ▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
- ▲ Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲ 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	Specification	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	

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.

▲ Materials prepared by users

Instruments

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

Reagents:

Double distilled water, Normal saline (0.9% NaCl)

⚠ Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the 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.

⚠ Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

Pre-assay preparation

▲ Reagent preparation

1. Bring all reagents to room temperature before use.

2. [Preparation of reagent 2 working solution:](#)

Dilute reagent 2 with double distilled water at a ratio of 1:79. Prepare the fresh needed amount before use and the prepared solution should be used up within 1 hour.

3. [Preparation of reagent 3 working solution:](#)

Dilute reagent 3 with double distilled water at a ratio of 1:4. Prepare the fresh needed amount before use and the prepared solution should be stored with shading light and used up within 1 hour.

4. [Preparation of 0.5 mmol/L standard solution:](#)

Dilute reagent 5 with double distilled water at a ratio of 1:19. Prepare the fresh needed amount before use and the prepared solution should be stored with shading light and used up within 1 hour.

5. [Preparation of determination working solution:](#)

Dilute reagent 1 with reagent 3 working solution at a ratio of 3:1. Prepare the fresh needed amount before use.

▲ Sample preparation

1. Serum (plasma) and urine samples:

Detect the sample directly. If the sample is turbidity, centrifuge at 10000 g for 10 min, then take the supernatant for detection.

2. Tissue sample:

Weigh the tissue accurately and add normal saline (0.9% NaCl) at a ratio of weight (g): volume (mL) =1: 9, homogenize the tissue in ice bath, centrifuge at 10000 g for 10 min at 4°C , then take the supernatant for measurement. If the supernatant is turbidity after centrifugation, centrifuge the supernatant repeated before use. Meanwhile, determine the protein concentration of supernatant.

3. Cell sample:

Collect the 1×10^6 cells, add 200 μ L normal saline (0.9% NaCl). Homogenize the cells sample with homogenizer on ice. Centrifuge the homogenized cells at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant.

▲ Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range 0.61- 49.37 U/L).

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).

Assay protocol

▲ Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S1'	S9	S9'	S17	S17'	S25	S25'	S33	S33'
B	B	B	S2	S2'	S10	S10'	S18	S18'	S26	S26'	S34	S34'
C	C	C	S3	S3'	S11	S11'	S19	S19'	S27	S27'	S35	S35'
D	D	D	S4	S4'	S12	S12'	S20	S20'	S28	S28'	S36	S36'
E	E	E	S5	S5'	S13	S13'	S21	S21'	S29	S29'	S37	S37'
F	F	F	S6	S6'	S14	S14'	S22	S22'	S30	S30'	S38	S38'
G	G	G	S7	S7'	S15	S15'	S23	S23'	S31	S31'	S39	S39'
H	H	H	S8	S8'	S16	S16'	S24	S24'	S32	S32'	S40	S40'

Note: A-H, standard wells; S1-S40, sample wells; S1'-S40', control wells.

▲ Detailed operation steps

1. The preparation of standard

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:

Number	Standard concentrations (μmol/L)	0.5 mmol/L standard solution (μL)	Double distilled water (μL)
A	0	0	200
B	0.1	40	160
C	0.15	60	140
D	0.2	80	120
E	0.3	120	80
F	0.35	140	60
G	0.4	160	40
H	0.5	200	0

2. The measurement of samples

(1) **Standard tube:** Take 20 μL of standard solution with different concentrations to the corresponding wells.

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

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

(2) Add 20 μL of reagent 2 working solution to each well.

(3) Add 80 μL of determination working solution to the standard wells and sample wells. Add 80 μL of reagent 1 to control wells.

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

(5) Add 100 μL of reagent 4 to each well.

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

▲ Summary operation table

	Standard well	Sample well	Control well
Standard solution with different concentrations (μ L)	20		
Sample (μ L)		20	20
Reagent 2 working solution (μ L)	20	20	20
Reagent 1 (μ L)			80
Determination working solution (μ L)	80	80	
Mix fully with microplate reader and incubate at 37°C for 10 min.			
Reagent 4 (μ L)	100	100	100
Mix fully and measure the OD value of each well at 405 nm with microplate reader.			

▲ Calculation

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample. The standard curve is: $y = ax + b$.

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 μ mol 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 cells sample

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

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

Note:

y: $OD_{Standard} - OD_{Blank}$ (OD_{Blank} is the OD value when the standard concentration is 0).

x: The concentration of standard.

a: The slope of standard curve.

b: The intercept of standard curve.

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

T: The time of reaction, 10 min.

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

f: Dilution factor of sample before test.

1000*: 1 mmol/L = 1000 μ mol/L.

Appendix I Data

▲ Example analysis

For 10% rat liver tissue homogenate, dilute for 5 times, take 20 μ L and carry the assay according to the operation table.

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:

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