# Fluoride Resistant Acid Phosphatase Activity Assay Kit

**Note:** Take two or three different samples for prediction before test.

**Operation Equipment:** Spectrophotometer//Microplate reader

**Cat No:** NA0132

**Size:**100T/48S

**Components:**

**Extract solution**:60 mL×1. Storage at 2-8℃.

**Reagent I**: 1.5 mL×1. Storage at 2-8℃.

**Reagent II**: 1.2 mL×1. Storage at 2-8℃.

**Reagent III**: powder×2,. Storage at -20℃. Before use, add 1mL of water, dissolve it fully. Unused reagents can be stored at -20℃ for 4 weeks.Avoid repeated freezing and thawing. One reagent can do 100T after dissolving. To prolong the use of the kit, therefore, one more powder is given.

**Reagent IV**: powder×1. Storage at 2-8℃. Before use, add 5.5mL of water, dissolve it fully. Unused reagents can be stored at 2-8℃ for 4 weeks.

**Reagent V** : 0.3 mL×1. Storage at 2-8℃. According to the ratio of reagent V: distilled water = 1:9 according to the number of samples before use.

**Reagent VI**: 1.2 mL×1. Storage at 2-8℃.

**Reagent VII**: 1.2 mL×1. Storage at 2-8℃.

**Reagent VIII**: 15 mL×1. Storage at 2-8℃.

**Standard:** 1 mL×1. Storage at 2-8℃. 5 μmol/mL phenol standard solution. Before use, take 100 μL of 5 μmol/mL phenol standard solution in an EP tube, add 300 μL of distilled water and mix thoroughly to make 1.25 μmol/mL phenol standard solution..

**Product Description:**

Fluoride resistant acid phosphatase (FRAP) is a type of acid phosphatase. FRAP is mainly found in the lysosomes of most cells, the prostate gland, brain, liver, spleen, and platelets.

The activity of fluoride-resistant acid phosphatase is not inhibited by fluoride ions, whereas the activity of other acid phosphatases is inhibited by fluoride ions. Under acidic conditions, the fluoride-resistant ion acid phosphatase catalyzes the production of p-nitrophenol from PNPP. The p-nitrophenol appears yellow under alkaline conditions and can be detected at 400 nm absorbance. The darker the yellow color of the product, the higher the activity of the fluorine-resistant ion acid phosphatase, and vice versa, the lower the enzyme activity.

**Reagents and Equipment Required but Not Provided:**

Spectrophotometer/microplate reader, centrifuge, water bath/incubator, adjustable pipette, Micro glass cuvettes/96 well plates, mortar/homogenizer/cell sonicator, ice and distilled water

**Procedure**

**I. Extraction of crude enzyme solution:**

a. Tissue

The ratio of tissue mass (g): the volume of Extract solution (mL) is 1: 5~10 (it is suggested to take about 0.1 g of tissue and add 1mL of Extract solution), ice-bath homogenate. Centrifuge at 8000 g for 10 minutes at 4℃, take the supernatant and placed on the ice for test.

b. Bacteria or cells

The ratio of bacteria/cell amount ($10^4$): the volume of Extract solution (mL) is 500~1000:1(it is suggested to take about 5 million bacteria/cells and add 1 mL of Extract solution). Bacteria/cell is split by ultrasonic (placed on ice, 200 W, work time 3 s，interval 10 s，repeat 30 times ). Centrifuge at 8000g for 10 minutes at 4℃, take the supernatant and placed on the ice for test.

c. Serum (plasma) sample: Detect sample directly. (If the solution is turbid, centrifuge to take the supernatant and then measure)

**II. Determination procedure**

a. Preheat the spectrophotometer/microplate reader 30 minutes, adjust wavelength to 400 nm, the spectrophotometer set zero with distilled water.

b. Then operate table.( Add the following reagents to the EP tube or 96 well plates)

| Reagent name （μL） | Test tube(T) | Control tube(C) | Standard tube(S) | Blank tube(B) |
|---|---|---|---|---|
| sample | 10 | 10 | - | - |
| Standard | - | - | 10 | - |
| distilled water | - | 10 | - | 10 |
| Reagent I | 10 | 10 | 10 | 10 |
| Reagent II | 10 | 10 | 10 | 10 |
| Reagent III | 10 | - | 10 | 10 |
| Reagent IV | 10 | 10 | 10 | 10 |
| Reagent V | 10 | 10 | 10 | 10 |
| Reagent VI | 10 | 10 | 10 | 10 |
| Reagent VII | 10 | 10 | 10 | 10 |
| | Reaction for 30 min at 37°C protected from light | | - | - |
| Reagent VIII | 120 | 120 | 120 | 120 |

After mixing, the absorbance at 400 nm was measured and recorded as At, Ac, As, and Ab. ΔAt = At-Ac, ΔAs= As- Ab. (Standard and standard blank tubes should be done only 1-2 times.)

**III. Calculation formula**

(1) Calculated by sample weight

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalytic production of 1 nmol phenol per gram of tissue per minute at 37°C .

FRAP activity（U/g mass）= (ΔAt×Cs÷ΔAs) ×Vs÷（W÷Vse×Vs）÷T×$10^3$×F=41.67×ΔAt÷ΔAs÷W×F

(2) Calculated by Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalytic production of 1 nmol phenol per mg protein of tissue per minute at 37°C .

FRAP activity（U/mg prot）=(ΔAt×Cs÷ΔAs) ×Vs÷（Cpr×Vs）÷T×$10^3$×F=41.67×ΔAt÷ΔAs÷Cpr×F

(3) Calculated by Cell amount

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalytic production of 1 nmol phenol per $10^4$ cells of tissue per minute at 37°C .

FRAP activity（U/$10^4$ cell）= (ΔAt×Cs÷ΔAs) ×Vs÷（N÷Vse×Vs）÷T×$10^3$×F=41.67×ΔAt÷ΔAs×F

(4) Calculated by the volume of culture medium

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalytic production of 1 nmol phenol every milliliter per minute at 37℃.

FRAP activity（U/mL）= (ΔAt×Cs÷ΔAs) ×Vs÷Vs÷T×$10^3$×F=41.67×ΔAt÷ΔAs×F

Cs: phenol standard solution，1.25 μmol/mL；Vs ：Sample volume in the reaction system ，0.01mL ；Vse ：T volume of extract，mL ；T ：Reaction time 30min ；Cpr ：Sample protein concentration mg/mL ；W ：Sample $10^3$：Unit conversion factor， 1μmol/mL=$10^3$nmol/mL；N：Total number of bacteria or cells；F：Sample dilution factor.

**Note:**

1. If At or ΔAt is greater than 2, the sample can be diluted with distilled water or the enzymatic reaction time at 37°C can be shortened; ΔAt is less than 0.01, the sample can be increased or the enzymatic reaction time at 37°C can be extended. The calculation formula was modified simultaneously for the final calculation.

**Experimental example:**

1. Weigh 0.1074g of rabbit liver tissue, add 1mL of extraction solution for ice bath homogenization, dilute the supernatant 2 times and follow the assay steps, use a 96-well plate to measure the calculated ΔAt= At - Ac= 0.485 - 0.067 = 0.418, ΔAs = As - Ab = 0.720 - 0.070 = 0.650, bring into the formula to calculate : FRAP activity (U/g mass)

   FRAP activity (U/g mass) = 41.67 × ΔAt ÷ ΔAs ÷ W × F = 499.013 U/g mass
2. Weigh 0.1057g of bamboo leaves, add 1mL of extract to ice bath and homogenize, follow the steps of the assay, use a 96-well plate to calculate ΔAt = At - Ac = 0.396 - 0.110 = 0.286, ΔAs = As - Ab= 0.720 - 0.070 = 0.650, bring into the formula to calculate.

   FRAP activity (U/g mass) = 41.67 × ΔAt ÷ ΔAs ÷ W × F = 173.461 U/g mass
3. 0.01 mL of sheep serum was aspirated and operated according to the assay procedure, and a 96-well plate was used to calculate ΔAt= At - Ac = 0.126 - 0.070 = 0.056, ΔAs= As - Ab = 0.720 - 0.070 = 0.650, which was brought into the formula to calculate.

   FRAP activity (U/mL) = 41.67 × ΔAt ÷ ΔAs × F = 3.590 U/mL

**Reference.:**

[1] Megat R, Wahab A, Dasor M M, et al. Crevicular tartrate resistant acid phosphatase activity and

rate of tooth movement under different continuous force applications[J]. African journal of pharmacy and pharmacology, 2011, 5(20):2213-2219.

[2] Natasˇa Mitic´, Mohsen Valizadeh, Eleanor W.W. Leung, et al. Human tartrate-resistant acid phosphatase becomes an effective ATPase upon proteolytic activation [J]. Archives of Biochemistry and Biophysics 439 (2005) 154–164.

**Related products:**

NA0718/NA0498 Tissue and blood alkaline phosphatase (AKP/ALP) activity assay kit

NA0719/NA0477 Tissue and blood acid phosphatase (ACP) activity assay kit

NA0137/NA0136 Tartrate resistant acid phosphatase activity assay Kit