

Fluoride Resistant Acid Phosphatase Activity Assay Kit

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

Operation Equipment: Spectrophotometer

Cat No: BC5450

Size: 50T/24S

Components:

Extract solution: 30 mL×1. Storage at 2-8°C.

Reagent I: 4 mL×1. Storage at 2-8°C.

Reagent II: 3.6 mL×1. Storage at 2-8°C.

Reagent III: powder×1. Storage at -20°C. Before use, add 3.5mL of water, dissolve it fully. Unused reagents can be stored at -20°C for 4 weeks. Avoid repeated freezing and thawing.

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

Reagent V : 0.5 mL×1. Storage at 2-8°C. According to the ratio of reagent V: distilled water = 1:9 according to the number of samples before use.

Reagent VI: 3.5 mL×1. Storage at 2-8°C.

Reagent VII: 3.5 mL×1. Storage at 2-8°C.

Reagent VIII: 40 mL×1. Storage at 2-8°C.

Standard: 1 mL×1. Storage at 2-8°C. 5 μmol/mL phenol standard solution. Before use, take 100 μL of 5 μmol/mL phenol standard solution in an EP tube, add 700 μL of distilled water and mix thoroughly to make 0.625 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, centrifuge, water bath/incubator, adjustable pipette, 1mL glass cuvette, 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°C, take the supernatant and placed on the ice for test.

b. Bacteria or cells

The ratio of bacteria/cell amount (10⁴): 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°C, 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 30 minutes, adjust wavelength to 400 nm, set zero with distilled water.

b. Then operate table.(Add the following reagents to the EP tube)

| Reagent name (μL) | Test tube(T) | Control tube(C) | Standard tube(S) | Blank tube(B) |
|--|--------------|-----------------|------------------|---------------|
| sample | 50 | 50 | - | - |
| Standard | - | - | 50 | - |
| distilled water | - | 50 | - | 50 |
| Reagent I | 50 | 50 | 50 | 50 |
| Reagent II | 50 | 50 | 50 | 50 |
| Reagent III | 50 | - | 50 | 50 |
| Reagent IV | 50 | 50 | 50 | 50 |
| Reagent V | 50 | 50 | 50 | 50 |
| Reagent VI | 50 | 50 | 50 | 50 |
| Reagent VII | 50 | 50 | 50 | 50 |
| Reaction for 30 min at 37°C protected from light | | | - | - |
| Reagent VIII | 600 | 600 | 600 | 600 |

After mixing, the absorbance at 400 nm was measured and recorded as At, Ac, As, and Ab. $\Delta At = At - Ac$, $\Delta As = As - Ab$. (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 .

$$\text{FRAP activity (U/g mass)} = (\Delta At \times C_s \div \Delta A_s) \times V_s \div (W \div V_{se} \times V_s) \div T \times 10^3 \times F = 20.83 \times \Delta At \div \Delta A_s \div W \times F$$

(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 .

$$\text{FRAP activity (U/mg prot)} = (\Delta A_t \times C_s \div \Delta A_s) \times V_s \div (C_{pr} \times V_s) \div T \times 10^3 \times F = 20.83 \times \Delta A_t \div \Delta A_s \div C_{pr} \times F$$

(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 10⁴ cells of tissue per minute at 37°C .

$$\text{FRAP activity (U/10}^4 \text{ cell)} = (\Delta A_t \times C_s \div \Delta A_s) \times V_s \div (N \div V_{se} \times V_s) \div T \times 10^3 \times F = 20.83 \times \Delta A_t \div \Delta A_s \times F$$

(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°C.

$$\text{FRAP activity (U/mL)} = (\Delta A_t \times C_s \div \Delta A_s) \times V_s \div V_s \div T \times 10^3 \times F = 20.83 \times \Delta A_t \div \Delta A_s \times F$$

Cs: phenol standard solution, 0.625 μmol/mL ; Vs: Sample volume in the reaction system, 0.05mL; Vse: The volume of extract, 1mL; T: Reaction time, 30min; Cpr: Sample protein concentration, mg/mL; W: Sample weight, g; 10³: Unit conversion factor, 1μmol/mL=10³nmol/mL; N: Total number of bacteria or cells; F: Sample dilution factor.

Note:

1. If At or ΔAt is greater than 1.2, 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 extract for ice bath homogenization, dilute the supernatant 2 times and operate according to the assay procedure, measure with a 1mL glass cuvette to calculate $\Delta A_t = A_t - A_c = 0.685 - 0.018 = 0.667$, $\Delta A_s = A_s - A_b = 0.582 - 0.031 = 0.551$, bring into Formula calculation.

$$\text{FRAP activity (U/g mass)} = 20.83 \times \Delta A_t \div \Delta A_s \div W \times F = 469.558 \text{ U/g mass}$$

2. Weigh 0.1057 g of bamboo leaves, add 1 mL of extract for ice bath homogenization, operate according to the determination procedure and measure with a 1 mL glass cuvette to calculate $\Delta A_t = A_t - A_c = 0.445 - 0.148 = 0.297$, $\Delta A_s = A_s - A_b = 0.582 - 0.031 = 0.551$, bring into the formula to calculate.

$$\text{FRAP activity (U/g mass)} = 20.83 \times \Delta A_t \div \Delta A_s \div W \times F = 106.223 \text{ U/g mass}$$

3. 0.05 mL of sheep serum was aspirated and operated according to the assay procedure, measured with a 1 mL glass cuvette to calculate $\Delta A_t = A_t - A_c = 0.084 - 0.028 = 0.056$, $\Delta A_s = A_s - A_b = 0.582 - 0.031 = 0.551$, brought into the formula to calculate.

$$\text{FRAP activity (U/mL)} = 20.83 \times \Delta A_{405} \div \Delta A_{540} \times F = 2.117 \text{ U/mL}$$

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] Natas`a 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:

BC2140/BC2145 Tissue and blood alkaline phosphatase (AKP/ALP) activity assay kit

BC2130/BC2135 Tissue and blood acid phosphatase (ACP) activity assay kit

BC5400/BC5405 Tartrate resistant acid phosphatase activity assay Kit