Pantothenic Acid (Vitamin B_5) Kit（Ready to use）

（Product No. GVT2004 ）

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1. Introduction

This product is a ready-to-use kit for Pantothenic Acid concentration by the microbiological assay technique .developed in accordance with the standard “GB5009.210-2023”, each product box contains 3 sets of reagents.

2. Principle of the Method

Pantothenic acid is an essential nutrient for the growth of *Lactiplantibacillus plantarum* (ATCC 8014). Under certain controlled conditions, *Lactiplantibacillus plantarum* suspension is inoculated into a culture medium containing the sample solution. After a period of incubation, the transmittance (or absorbance) is measured. The pantothenic acid content in the sample is then calculated based on the standard curve of pantothenic acid content versus transmittance (or absorbance).

3. Reagents provided

Pantothenic acid Standards（Freeze-dried）	3 vials
Pantothenic acid Bacterial Ball（Freeze-dried）	3 vials
Pantothenic acid Medium Base (20 mL)	3 vials
Pantothenic acid Medium Additive (Freeze-dried)	3 vials
Sterile Water(30mL)	3 vials
Sterile 96-well Microplate individually packed	3 plates
Sealing Film	3 pieces

4. Storage condition:

Store in dark place 2-8℃ for a year

5. Materials required but not provided

5.1 Ultra-clean workstation

5.2 Constant temperature incubator, 36℃ ±1℃

5.3 Microplate reader (540nm~610 nm)

5.4 Autoclave

5.5 Ultrasonic oscillator

5.6 Vortex mixer

5.7 Pipette and sterile tips, 20-200 μL, 100-1000 μL

5.8 conical flask and volumetric flask

5.9 Pipette centrifuge tubes: 1.5 mL or 2 mL,15 mL tubes should have screw tops

5.10 Sterile syringes and 0.22 μm sterile filter

membrane

6. Assay medium preparation (aseptic procedure)

6.1 Preparation of Pantothenic Acid Assay Medium: Pipette 1.0mL of the basic pantothenic acid assay medium into the additive of the Pantothenic Acid Assay Medium and mix well.
6.2 Non-inoculation of standard 0 control tube medium: 200μL of the aforementioned medium was taken and placed in a 1.5mL sterile centrifuge tube.
6.3 Inoculation of Pantothenic Acid Assay Medium: take 1 vial of pantothenic acid bacterial ball, add it to the prepared Pantothenic Acid Assay Medium (6.1), mix well and then use.

7. Preparation of standard tubes (aseptic operation)

Accurately pipette 1.5mL of sterile water into the pantothenic acid Standard, dissolve and mix thoroughly. Then take 1 mL of the solution and add it to 4 mL of sterile water, mix well, and this is the Pantothenic Acid Standard working solution. Take 11 sterile 1.5 mL(or 2 mL) centrifuge tubes and prepare a series of standard solutions with varying concentrations according to Table 1

Table 1 Preparation of standard curves

Number	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11
pantothenic acid content /ng	0.00	2.00	4.00	6.00	8.00	10.0	12.0	14.0	16.0	18.0	20.0
Sterile Water /μL	500	450	400	350	300	250	200	150	100	50	0
Standard solution /μL	0	50	100	150	200	250	300	350	400	450	500

8. Preparation of Sample Tubes

8.1 The sample is prepared in accordance with step "20.1-20.3" in GB 5009.210-2023. "
8.2 The prepared sample diluent is filtered and sterilized under sterile conditions using a sterile aqueous phase filter membrane (0.22μm). And a series of sample tubes are prepared in the order specified in Table 2.

Table 2: Preparation of specimen tubes

Sample tube number	1	2	3	4
Sterile Water /μL	100	200	300	400
Sample solution /μL	400	300	200	100

9. Detection steps (aseptic operation)

9.1Take out the sterile 96-well microplate, record the well positions, and conduct 2~3 parallel tests for the standard solution using separate wells. Additionally, prepare one well and add 150 μL of sterile water to serve as a non-inoculated standard 0 control

9.2 Add 150 μL of prepared inoculated pantothenic acid bacterial ball assay medium (6.3) to each well, and add 150μL of uninoculated pantothenic acid bacterial ball assay medium(6.2) to the standard 0 control tube
9.3 Transfer 150 μL of the standard series tube or sample series tube into the designated well.
9.4 Seal the wells on the strip with a sealing film and press the film to ensure that all wells are adequately sealed (The edge part of the wells section should be fully sealed, with special attention paid to it)

10. Incubation

Incubate at 36°C ± 1°C for 32-40 h in an incubator, avoiding light.

11.Measurement

11.1 Take out the plate from the incubator and press the sealing film again to ensure that all wells are adequately sealed. Shake the plate upside down repeatedly to mix the microorganisms well.
11.2 Remove the sealing film diagonally and puncture the air bubbles on the surface of each well with a needle.
11.3 Measure the absorbance value at 550 nm with a microplate reader.
Note: The assay should be terminated in any of the following situations: significant bacterial growth is observed in the inoculated 0 control tube; the absorbance value of the standard 0 tube is >0.3 compared with the 0 control tube; the change in absorbance value of the standard series tubes is <0.4 compared with the standard 0 tube.

12. Data analysis

Analyze the results according to 'Part 21: Expression of Analytical Results' in the national standard GB 5009.259-2023.
12.1 Standard curve: Using the pantothenic acid concentration of the standard series tubes as the abscissa and the average transmittance (or absorbance value) of each standard point as the ordinate, a standard curve is plotted and a four-parameter curve equation is fitted
12.2 Result calculation:
In sample diluent, pantothenic acid concentration and average value are calculated according to formulas (1), (2):

$$c_i = c_x \times \frac{V_5}{V_x} \quad \cdots\cdots\cdots(1)$$

$$\bar{c} = \frac{\sum_{i=1}^{n} c_i}{n}$$

·····(2)

C_i— pantothenic acid concentration in the sample diluent, ng/mL;

C_x—According to the standard curve to calculate samples series of pantothenic acid concentration in tubes, ng/mL;

$\frac{V_5}{V_x}$ —The total volume sample tube/added sample diluent;

$\bar{C}$— Pantothenic acid concentration in sample diluent.ng/mL;

$\sum_{i=1}^{\bar{c}} c_i$ —The sum of the sample diluent concentration calculated by the effective tube, ng/mL;

n—Number of effective tubes.

The pantothenic acid content of the sample using the direct extraction method is calculated according to formula (3):

$$X = \frac{\bar{c} \times V \times f}{m} \times \frac{100}{10^6}$$

·····(3)

X—Pantothenic acid content in the sample. mg/100 g;

$\bar{C}$ —Average folate concentration in sample diluent ng/mL;

V— Sample extract volume volume mL;

f— Dilution factor of sample extract;

m—Sample mass g;

$\frac{100}{10^6}$ —Unit conversion coefficient

The pantothenic acid content of the sample using the enzymatic extraction method is calculated according to formula (4):

$$X = \frac{(\bar{c} - \bar{c}_0) \times V}{m} \times \frac{V_2 \times V_4}{V_1 \times V_3} \times f \times \frac{100}{10^6}$$

·····(4)

X—Pantothenic acid content in the sample, μg/100 g ;

$\bar{C}$—Average pantothenic acid concentration in sample diluent, ng/mL;

$\bar{C}_0$—Average concentration of pantothenic acid in enzyme blank solution ,ng/mL;

V_2、V_4—Fixed volume after adjusting pH value,mL;

V_1、V_3—Adjust pH when inhaled sample extraction fluid volume,mL;

f— Dilution factor of sample extract;

m—sample mass g;

$\frac{100}{10^6}$ —Unit conversion coefficient

The value retains three significant digits.

For laboratory use in industry or R&D purpose. Not for drug, household or other uses.

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