Pantothenic Acid(VitaminB$_5$) Kit(ready-to-use)(GB)

（Product No. GVT1004 ）

GFAD[02]1.23

1. Introduction

This product is an ready-to-use kit for Pantothenic acid detection by tubes method, developed in accordance with the standard “GB5009.210-2023”, each product box contains 2 sets of reagents and each reagent preparation in tubes (50) .

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）	2 vials
Pantothenic Acid Bacterial Ball（Freeze-dried）	2 vials
Pantothenic Acid Medium Base	250 mL×2
Pantothenic Acid Medium Additive (Freeze-dried)	2 vials

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, 37℃ ±1℃

5.3 Uv-vis spectrophotometer

5.4 Autoclave

5.5 Ultrasonic oscillator

5.6 Vortex mixer

5.7 Sterile tubes and racks

5.8 Pipette and sterile tips, 10-100 μL, 100-1000 μL, 500-5000μL

5.9 Sterile water

5.10 Sterile centrifugal tubes with cover: 15 mL,50 mL

5.11 Sterile syringes and 0.22 μm sterile filter membrane

6. Assay medium preparation (aseptic procedure)

6.1 **Preparation of Pantothenic Acid Assay Medium**

6.1.1 Add 1.1mL sterile water into **Pantothenic Acid Medium Additive** and mix for 3 minutes, complete mixing, and then add 1mL to 250mL **Pantothenic Acid Medium Base**, and mix well.

6.1.2 Take 1 vial of of **Pantothenic Acid Bacterial Ball** add into (6.1.1)the assay medium,mix well.

6.2 **Preparation of standard solution**

6.2.1 Pantothenic acid standard solution：Add 5ml sterile water to **Pantothenic Acid Standard**, dissolve and mix well.

6.2.2 Pantothenic acid standard working solution：Accurately take 1mL of Pantothenic Acid Standard solution(6.2.1) into 9ml of sterile water, mix and use.Ready-to-use.

7. Preparation of Samples

According to the standard for sample processing and dilution.

8. Preparation of standard tubes (aseptic operation)

8.1 Standard tubes

Add sterile water, Pantothenic acid working solution（6.2.2）, and pantothenic acid medium(6.1.2) to sterile test tubes according to Table 1.

Table 1-1 Preparation of standard curves

Number	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11
Sterile Water /mL	5.00	4.95	4.90	4.85	4.80	4.75	4.70	4.65	4.60	4.55	4.50
Pantothenic Acid Working Solution /mL	0.0	0.05	0.10	0.15	0.20	0.25	0.30	0.35	0.40	0.45	0.50
Medium/mL	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00

The standard tubes,2-3 sets should be prepared.

8.2 Assay tubes and Enzyme blank tubes

The prepared sample diluent was filtered by g0.22μm sterile filter membrane,and then add sterile water, sterile sample solution and medium in sterile test tube according to Table 2.Mix well.

Table 2: Preparation of assay tubes

Sample tube number	S1	S2	S3	S4
Sterile Water /mL	4.00	3.00	2.00	1.00
Sample solution /mL	1.00	2.00	3.00	4.00
Medium/mL	5.00	5.00	5.00	5.00

9. Incubation

Incubate at 36°C ± 1°C for 24-48 h in an incubator, avoiding light.Be sure to grow the end.

10. Measurement

The cultured standard tubes, the assay tubes and the enzyme blank tubes were used a vortex shaker ,mix well. A microplate reader or a cuvette with thickness 1cm was used for determination at 550nm.

11. Data analysis

Analyze the results according to the national standard GB 5009.210-2023.

11.1 **Standard curve**: Using the pantothenic acid content of the standard series tubes as the abscissa and the transmittance (or absorbance value) of each standard point as the ordinate, a standard curve is plotted.

11.2 **Result calculation:**

In sample diluent, pantothenic acid concentration and average value are calculated according to formulas (1), (2):

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

$$\bar{c} = \frac{\sum_{i=1}^{n} c_i}{n} \quad \cdots\cdots\cdots(2)$$

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):

$$X = \frac{\bar{c} \times V \times f}{m} \times \frac{100}{10^6} \quad \cdots\cdots(3)$$

X—Pantothenic acid content in the sample: μg/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):

$$X = \frac{(\bar{c} - \bar{c}_0) \times V}{m} \times \frac{V_2 \times V_4}{V_1 \times V_3} \times f \times \frac{100}{10^6} \quad \cdots\cdots(4)$$

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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