Biotin(Vitamin B_7) Kit（Ready to use）

（Product No. GVT2003）

GBTN[01]10.23

Introduction

This product is a ready-to-use kit for Biotin detection by the microbiological assay technique developed in accordance with the standard “GB5009.259-2023”， each product box contains 3 sets of reagents.

2. Principle of the Method

Biotin 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 biotin content in the sample is then calculated based on the standard curve of the biotin content versus transmittance (or absorbance).

3. Reagents provided

Biotin Standards（Freeze-dried）	3 vials
Biotin Bacterial Ball（Freeze-dried）	3 vials
Biotin Medium Base (20 mL)	3 vials
Biotin 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~550nm or 610nm～630nm)

5.4 Autoclave

5.5 Ultrasonic oscillator

5.6 Vortex mixer

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

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 Biotin Assay Medium: Pipette 1.0mL of the basic biotin assay medium into the additive of the Biotin Acid Assay Medium, and mix well.

6.2 Non-inoculation of standard blank tube medium: 200 μL of the aforementioned medium was taken and placed in a 1.5mL sterile centrifuge tube.

6.3 Inoculation of biotin assay medium: take 1 vial of of biotin bacterial ball, add it to the prepared folic acid assay medium (6.1), mix well and then use.

7. Preparation of standard tubes (aseptic operation)

Accurately pipette 1.5 mL of sterile water into the Biotin 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 biotin standard working solution.

Take 10 sterile 1.5 mL centrifuge tubes and prepare a series of standard solutions with varying concentrations according to Table 1

Table 1-1 Preparation of standard curves

Number	UN	IN	S1	S2	S3	S4	S5	S6	S7	S8
Biotin content /ng	0.00	0.00	0.10	0.20	0.30	0.40	0.50	0.60	0.80	1.00
Sterile Water /μL	1000	1000	900	800	700	600	500	400	200	0
Standard solution /μL	0	0	100	200	300	400	500	600	800	1000

8. Preparation of Sample Series Tubes

8.1 The sample is prepared, extracted, and diluted according to the corresponding steps "12.2" outlined in GB 5009.259-2023.

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	800	600	400	200
Sample solution /μL	200	400	600	800

9. Detection steps (aseptic operation)

9.1 Take out the sterile 96-well microplate and record the position of each well. Conduct 3 parallel tests for the standard solution and the sample diluent using separate wells. Additionally, prepare one well and add 150 μL of sterile water to serve as a non-inoculated standard blank control (UN)

9.2 Add 150 μL of prepared inoculated Biotin Assay Medium (6.3) to each well, and add 150 μL

of uninoculated biotin bacterial ball assay medium(6.2) to the standard blank control tube
9.3 Transfer 150 μL of the standard series tube and 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 32-40h 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 540nm ~550nm or 610nm ~630nm with a microplate reader.
Note: The culture medium in the non-inoculated standard blank control (UN) absorbance should be less than 0.1, otherwise the assay will not be effective

12. Data analysis

Analyze the results according to 'Part 13: Expression of Analytical Results' in the national standard GB 5009.259-2023.
12.1 Standard curve: Using the folic acid concentration of the standard series tubes as the abscissa and the average transmittance (or absorbance) of each standard point as the ordinate, a standard curve is plotted.
12.2 Result calculation:
Analyze the results according to 'Part 13: Expression of Analytical Results' in the national standard GB 5009.89-2023.
12.1 Standard curve: Using the biotin concentration of the standard series tubes as the abscissa and the average transmittance (or absorbance) of each standard point as the ordinate, a standard curve is plotted.
12.2 Result calculation:
Biotin concentration in the sample diluent: (1)

$$\rho_x = \frac{c_x}{V_x} \quad \cdots\cdots\cdots(1)$$

ρ_x—Biotin concentration in the sample diluent, ng/mL;
c_x—Biotin content in the sample series tube obtained from the standard curve, ng;
v_x—Volume of the sample diluent aspirated when preparing the sample series tube, mL;

The biotin content of the sample is calculated according to formula (2) :

$$X = \frac{\rho \times V}{m} \times \frac{V_1}{V_2} \times f \times \frac{100}{1\,000} \quad \cdots\cdots\cdots(2)$$

X— Biotin content in the sample: μg/100 g (mL);
ρ— Average biotin concentration in sample diluent ng/mL;
V— Sample extract volume volume mL;
m— Sample mass g;
V_1—Constant volume before filtration
V_2—Constant volume after filtration
f— Dilution factor of sample extract;
100—Unit conversion coefficient
1000—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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