

Biotin(Vitamin B₇) Kit(ready-to-use)(GB)

(Product No. GVT1003)

GFAD[02]1.23

1. Introduction

This product is an ready-to-use kit for Biotin detection by tubes method, developed in accordance with the standard “GB5009.259-2023” , each product box contains 2 sets of reagents and each reagent preparation in tubes (50) .

2. Principle of the Method

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

3. Reagents provided

Biotin Standards (Freeze-dried)	2 vials
Biotin Bacterial Ball (Freeze-dried)	2 vials
Biotin Medium Base	250 mL×2
Biotin Medium Additive (Freeze-dried)	2 vials

4. Storage condition:

Store in dark place 2-8°C for a year.

5. Materials required but not provided

5.1 Ultra-clean workstation	5.7 Sterile tubes and racks
5.2 Constant temperature incubator, 37°C ±1°C	5.8 Pipette and sterile tips, 10-100 μL, 100-1000 μL, 500-5000μL
5.3 Uv-vis spectrophotometer	5.9 Sterile water
5.4 Autoclave	5.10 Sterile centrifugal tubes with cover: 15 mL,50 mL
5.5 Ultrasonic oscillator	5.11 Sterile syringes and 0.22 μm sterile filter membrane
5.6 Vortex mixer	

6. Assay medium preparation (aseptic procedure)

6.1 Preparation of Biotin Assay Medium

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

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

6.2 Preparation of standard solution

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

6.2.2 Biotin standard working solution: Accurately take 2mL of Biotin Standard solution(6.2.1) into 8ml 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, biotin working solution (6.2.2) , and biotin 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
Sterile Water /mL	5.0	4.95	4.90	4.85	4.80	4.75	4.70	4.60	4.50
Biotin Working Solution /mL	0.0	0.05	0.10	0.15	0.20	0.25	0.30	0.40	0.50
Medium/mL	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

The prepared sample diluent was filtered by g0.22μm sterile filter membrane,and then add sterile water, sterile sample solution and medium(6.1.2.) 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 37°C ± 1°C for 44-48 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 5009.259-2023.

11.1 **Standard curve:** Using the biotin 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:

The corresponding content of biotin (C_x) in the sample is obtained from the standard curve. If 2 out of 3 test tubes of each sample fall between 0.1-1ng and the absorbance value deviation of each tube is less than 10%, the results are calculated according to formulas (1) :

Biotin concentration in the sample diluent:

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

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 using the direct extraction method is calculated according to formula (2):

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

X —Biotin content in the sample: $\mu\text{g}/100\ \text{g}$ (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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