Folic Acid(Vitamin B_9) Kit(ready-to-use)(GB)

（Product No. GVT1001 ）

GFAD[02]1.23

1. Introduction

This product is an ready-to-use kit for Folic acid detection by tubes method, developed in accordance with the standard “GB5009.211-2022”, each product box contains 2 sets of reagents and each reagent preparation in tubes (50) .

2. Principle of the Method

Folic acid is an essential nutrient for the growth of *Lactobacillus rhamnosus* (ATCC 7469). Under certain controlled conditions, *Lactobacillus rhamnosus* suspension is inoculated into a culture medium containing the sample solution. After a period of incubation, the transmittance (or absorbance) is measured. The folic acid content in the sample is then calculated based on the standard curve of folic acid content versus transmittance (or absorbance).

3. Reagents provided

Folic Acid Standards（Freeze-dried）	2 vials
Folic Acid Bacterial Ball（Freeze-dried）	2 vials
Folic Acid Medium Base	250 mL×2
Folic 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 Folic Acid Assay Medium**

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

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

6.2 **Preparation of standard solution**

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

6.2.2 Folic acid standard working solution: Accurately take 2mL of Folic Acid 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, folic acid working solution（6.2.2）, and folic 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
Sterile Water /mL	5.000	4.975	4.950	4.900	4.850	4.800	4.750	4.700	4.600	4.500
Folic Acid Working Solution /mL	0.0	0.025	0.050	0.100	0.150	0.200	0.250	0.300	0.400	0.500
Medium/mL	5.000	5.000	5.000	5.000	5.000	5.000	5.000	5.000	5.000	5.000

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
Sterile Water /mL	4.00	3.00	2.00
Sample solution /mL	1.00	2.00	3.00
Medium/mL	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 540nm.

Note: Measure the absorbance at 540 ~ 610 nm.

11. Data analysis

Analyze the results according to the national standard GB 5009.211-2022.

11.1 **Standard curve**: Using the folic 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:**

The corresponding content of folic acid (Cx) in the sample or enzyme blank series tubes is obtained from the standard curve. If the folic acid content in two of the three sample series tubes is within the range of 0.10 ng to 0.80 ng, and the deviation in the folic acid content of each tube is less than 10% per milliliter of the sample extract solution, the results are calculated according to formulas (1), (2), and (3):

Folic acid concentration in the sample diluent:

$$c = \frac{c_x}{V_x} \qquad \cdots\cdots\cdots(1)$$

C — Folic acid concentration in the sample diluent, ng/mL;

Cx — Folic acid content in the sample series tube obtained from the standard curve, ng;

Vx — Volume of the sample diluent aspirated when preparing the sample series tube, mL.

The folic acid content of the sample using the direct extraction method is calculated according to formula (2):

$$X = \frac{\bar{c} \times V \times f}{m} \times \frac{100}{1\,000} \qquad \cdots\cdots\cdots(2)$$

X—Folic acid content in the sample: μg/100 g (mL);

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

V— Sample extract volume volume mL;

f— Dilution factor of sample extract;

m—sample mass g;

$\frac{100}{1\,000}$ —Unit conversion coefficient

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

$$X = \frac{(\bar{c} \times f - \bar{c}_0) \times V}{m} \times \frac{100}{1\,000} \quad \cdots \quad \cdots\cdots(3)$$

$\bar{C}_0$—Average concentration of folic acid in enzyme blank solution (ng/mL)

The meaning of X、c、f、V、m、$\frac{100}{1\,000}$ in the formula is the same as that in formula (2).

Under repeated conditions, the arithmetic mean value of two independent measurement results is obtained, and 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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