Folic Acid (Vitamin B_9) Kit（Ready to use）

（Product No. GVT2001 ）

GFAD[01]09.22

1. Introduction

This product is a ready-to-use kit for Folic acid concentration by the microbiological assay technique .developed in accordance with the standard “GB5009.211-2022”， each product box contains 3 sets of reagents.

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）	3 vials
Folic Acid Bacterial Ball（Freeze-dried）	3 vials
Folic Acid Medium Base (20 mL)	3 vials
Folic 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 (540~610 nm)

5.4 Autoclave

5.5 Ultrasonic oscillator

5.6 Vortex mixer

5.7 Pipette and sterile tips, 10-100 μL, 100-1000 μL

5.8 conical flask and volumetric flask

5.9 Pipette centrifuge tubes: 1.5 mL, 15 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 Folic Acid Assay Medium: Pipette 1.0mL of the basic folic acid assay medium into the additive of the Folic Acid Assay Medium, dissolve for 3 minutes to ensure complete mixing, and then transfer the entire mixture into 20mL of the basic Folic Acid Assay Medium, and mix well.

6.2 Non-inoculation of standard 0 control tube medium: 200 μL of the aforementioned medium was taken and placed in a 1.5mL sterile centrifuge tube, which serves as the medium for the non-inoculated standard 0 control tube

6.3 Inoculation of folic acid assay medium: take 1 vial of of folic acid 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)

This product provides the following two methods for preparing standard series tubes. "Method 1" is consistent with the national standard procedure, while "Method 2" omits the step of diluting the standard working solution. Both methods yield standard series tubes with identical folic acid content, and you can choose either method depending on the situation.

Method 1: Accurately pipette 1.5 mL of sterile water into the Folic 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 folic acid standard working solution. Take 10 sterile 1.5 mL centrifuge tubes and prepare a series of standard solutions with varying concentrations according to Table 1-1.

Table 1-1 Preparation of standard curves

Number	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
Folic acid content /ng	0.00	0.05	0.10	0.20	0.30	0.40	0.50	0.60	0.80	1.00
Standard solution /μL	0	50	100	200	300	400	500	600	800	1000
Sterile Water /μL	1000	950	900	800	700	600	500	400	200	0

Method 2: Accurately pipette 1.5 mL of sterile water into the folic acid standard, dissolve and mix thoroughly. Then take 10 sterile 1.5 mL centrifuge tubes and prepare a series of standard solutions with varying concentrations according to Table 1-2.

Table 1-2 Preparation of standard curves

Number	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
Folic acid content /ng	0.00	0.05	0.10	0.20	0.30	0.40	0.50	0.60	0.80	1.00
Standard solution /μL	0	10	20	40	60	80	100	120	160	200
Sterile Water /μL	1000	990	980	960	940	920	900	880	840	800

8. Preparation of Sample Series Tubes

8.1 The sample is prepared, extracted, and diluted according to the corresponding steps "6.1 to 6.3" outlined in GB 5009.211-2022.
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	S1	S2	S3
Sample solution /μL	100	200	300
Sterile Water /μL	400	300	200

9. Detection steps (aseptic operation)

9.1 Take out the sterile 96-well microplate, record the position of the wells. Conduct 2~3 parallel tests for the standard solution using separate wells. Perform 2 parallel tests for each gradient of the sample diluent in separate wells. Additionally, prepare1 well and add 150 μL of sterile water as a non-inoculated standard 0 control tube (containing 0.00 ng of folic acid).
9.2 Add 150 μL of prepared inoculated Folic Acid Bacterial Ball assay medium (6.3) to each well, and add 150 μL of uninoculated Folic Acid Bacterial Ball assay medium (6.2) to the standard 0 control tube
9.3 Transfer 150 μL of the standard series tube (7) or sample series tube (8.2) 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 40-48 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 540 nm with a microplate reader.
If the 0 control well appears turbid, it indicates possible contamination by miscellaneous bacteria, and the test needs to be redone.
Note: Measure the absorbance at 540 nm ~ 610 nm.

12. Data analysis

Analyze the results according to 'Part 7: Expression of Analytical Results' in the national standard GB 5009.211-2022.

12.1 Standard curve: Using the folic acid content 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:

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;

C_x — Folic acid 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.

Note: When 100μL, 200μL, and 300μL are aspirated using the microplate method, the corresponding Vx values are 1mL, 2mL, and 3mL, respectively.

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;

$\frac{100}{1000}$ —Unit conversion coefficient

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

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

$\bar{C}_0$—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).

12.3 Description

The result of the calculation retains three significant figures.

Note: The folic acid content of liquid samples can also be measured in micrograms per 100 milliliters (μg/100mL).

Refer to national standards for precision, detection limit, and quantification limit.

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

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