Folic Acid Assay Kit

(Product No. VT3101)

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1. Introduction

The test kit uses the specificity of Lac*tobacillus casei spp. rhamnosus* ATCC 7469 for folic acid to determine the amount of folic acid through the turbidity formed by bacterial growth in samples containing folic acid.

2. Principle of the Method

The growth intensity (turbidity) of *Lactobacillus rhamnosus* is linearly related to the amount of folic acid in the medium containing all nutrients except folic acid. The medium, *Lactobacillus rhamnosus* and the sample (or standards) were added to the 96-well microplate and *Lactobacillus rhamnosus* will grow until folic acid is depleted. A standard curve is plotted using the turbidity of the bacteria after incubation in standards against the different concentrations of the standards, and the amount of folic acid in the sample is obtained by measuring the turbidity of *Lactobacillus rhamnosus* in the samples on the standard curve.

3. Product properties

Procedure time: operation time: 1h; incubation time: 44-48h
Range: 0.1-1.0 μg/100 g(mL)
Recovery: 80-120%
Intra-batch variation: <10%
Inter-batch variation: <10%
Storage condition: 1 year shelf life under 2-8℃ storage

4. Reagents provided

Folic Acid Standards	3 vials
Folic Acid Test Bacterial Ball	3 vials
Folic Acid Medium	3 vials
1X Folic Acid Buffer (50 mL/vial)	3 vials
20X Folic Acid Detection Buffer (30 mL/vial)	1 vial
Folic Acid Protectant	3 vials
Sterile Water(10mL/bottle)	3 vials
Sterile 96-well Microplate individually	3 plates

packed	
Sealing Film	3 pieces

5. Materials required but not provided

5.1 Ultra-clean workstation	5.7 Pipette and sterile tips, 20-200 μL, 100-1000 μL
5.2 Microplate reader (550 or 630 nm)	5.8 15mL and 50mL sterile centrifuge tubes with screw cap and 1.5 mL or 2 mL sterile centrifuge tubes
5.3 Constant temperature incubator, 36℃±1℃	5.9 Sterile syringes and 0.22 μm sterile filter membrane
5.4 Autoclave	5.10 Distilled water
5.5 Water bath, 95℃	5.11 Graduated cylinder and 300 mL conical flask
5.6 Vortex mixer	

6. Reagent preparation

6.1 Folic Acid Protective Solution: In ultra-clean workstation, take 1 vial of Folic Acid Protectant, add 10 mL of 1X Folic Acid Buffer and dissolve thoroughly to make Folic Acid Protective Solution (Prepare it fresh when needed).
Note: Folic Acid Protectant can also be dissolved in 10 mL of distilled water.

6.2 Sample Preparation Solution (20 mL/sample): Dilute 20X Folic Acid Detection Buffer with distilled water to get 1X Folic Acid Detection Working Buffer. Dilute Folic Acid Protective Solution in 6.1 with 1X Folic Acid Detection Working Buffer to create Sample Preparation Solution, the ratio is Folic Acid Protective Solution: 1X Folic Acid Detection Working Buffer = 1:50 (Prepare it fresh when needed).
Note: Calculate the volume of Sample Preparation Solution required based on the number of samples to be tested. Each 96-well plate can generally accommodate the test of 8 samples and it requires 160 mL of Sample Preparation Solution, so transfer 10 mL of 20X Folic Acid Detection Buffer into a clean triangular flask, add 190 mL of distilled water and mix well to obtain 200 mL of 1X Folic Acid Detection Working Buffer. Then add 4 mL of Folic Acid Protective Solution in 6.1 inside the 200 mL of 1X Folic Acid Detection Working Buffer to obtain the needed Sample Preparation Solution.

6.3 Standard/sample Diluent: In the ultra-clean workstation, remove bacteria by filtering

the Folic Acid Protective Solution in 6.1 through a 0.22 μm filter membrane and then add the filtered Folic Acid Protective Solution to 1X Folic Acid Buffer at a ratio of filtered Folic Acid Protective Solution : 1X Folic Acid Buffer = 1:50.

7. Sample preparation of milk powder

7.1 Weigh 1 g (to the accuracy of 0. 01 g) of milk powder in a 50 mL centrifuge tube and add 20 mL of Sample Preparation Solution, **corresponding to an extraction dilution factor of 20, which is already included in the standard curve.** Mix it well and put the tube in a water bath at 95°C for 30 min, during which mixing it periodically on a vortex (at least 5 times), then quickly cool it to below 30°C in ice water.

The following operations need to be carried out in the ultra-clean workstation:

7.2 Filter the cooled extract through a 0.22 μm filter membrane into a 2 mL sterile centrifuge tube to create a sterile sample solution.

7.3 Dilute the sterile sample extract with Standard/sample diluent in 6.3 to folic acid concentrations of approximately 0.1, 0.2 and 0.4 μg/100 g (mL).

Example of sample preparation

For example, to test the folic acid concentration in an infant milk powder sample labeled with 80 μg/100 g (mL) folic acid inside, weight 1 g of sample in a 50 mL centrifuge tube and add 20 mL Sample Preparation Solution in 6.2. Put the tube in water bath at 95℃ for 30 min for extraction, and then quickly cool it down to below 30℃. Filter the cooled extract through a 0.22 μm filter membrane to remove bacteria. Dilute the filtered sample extract 800 times, 400 times and 200 times to obtain the final concentrations of folic acid approximately at 0.1 μg/100 g (mL), 0.2 μg/100 g (mL) and 0.4 μg/100 g (mL) respectively. The dilution method is listed as follows:

Sample dilution times	Dilution protocol
①10 times	900μL **of Standard/sample Diluent**+100μL of **sample extract**
②100 times	900 μL **Standard/sample Diluent** +100 μL of **① solution**
③200 times	200 μL **Standard/sample Diluent** +200 μL**② solution**
④400 times	300 μL **Standard/sample Diluent** +100 μL**② solution**
⑤800 times	700 μL **Standard/sample Diluent** +100 μL**② solution**

Note: Samples should be fully mixed after each dilution step and sample extracts must be used

on the same day and stored in dark place. In case of unknown samples, two folic acid concentrations should be assumed and processed separately as above, with the two assumed values being adjacent orders of magnitude, following the steps above.

8. Method procedure

8.1 Folic Acid Standards preparation (to be carried out in an ultra-clean workstation)

Take 1 vial of lyophilized folic acid standard and add 2.5 mL of Standard/sample Diluent to prepare a standard solution of folic acid. Take 8 sterile 1.5 mL centrifuge tubes and prepare a series of standard solutions from 0.1 to 1.0 μg/100 g (mL) according to the table below:

μg/100 g(mL)	Volume of standard solution (μL)		Volume of Standard/sample Diluent (μL)		Total volume μL
Blank: 0	0	+	500	=	500
Standard 1: 0.1	50	+	950	=	1000
Standard 2: 0.2	100	+	900	=	1000
Standard 3: 0.3	150	+	850	=	1000
Standard 4: 0.4	200	+	800	=	1000
Standard 5: 0.6	300	+	700	=	1000
Standard 6: 0.8	400	+	600	=	1000
Standard 7: 1.0	500	+	500	=	1000

Note: Standard solutions should be prepared fresh when needed and they can not be stored.

8.2 Preparation of folic acid Medium Solution

1) Pour one bottle of sterile water provided in the kit into one vial of folic acid medium, and then tighten the cap and shake it until dissolved.
2) Put the folic acid medium vial in water bath at 95°C for 5 min and shake it 2-3 times during this time, and then quickly cool it in ice water to below 30°C.
3) In the ultra-clean workstation, filter the folic acid medium solution through a sterile 0.22 μm filter membrane into a 15 mL sterile centrifuge tube. Each vial of folic acid medium is sufficient for 1 microplate of 96-well.

8.3 and 8.4 below need to be carried out in the ultra-clean workstation:

8.3 Preparation of Folic Acid Test Bacterial Solution

Dissolve 1 vial of folic acid bacterial ball in the filtered folic acid medium solution in 8.2, and then tighten the cap and shake it until fully mixed.

8.4 Assay procedure

1) Determine the number of microwell strips required to test the desired number of samples plus the number of wells needed for standards, considering that each sample and standard need be tested in triplet. Insert the appropriate number of strips in the holder, and record the position of the wells to create a layout. Immediately reseal the unused strips in the bag together with the desiccant bag provided and store in 2-8℃.

2) Add 100 µL of **folic acid test bacterial solution** in 8.3 in each well.

3) Add 100 µL of Blank Standard (zero standard) to wells A1, A2 and A3; add 100 µL of each standard solution (Standard 1-7) to wells B1, B2 and B3, C1, C2 and C3 -- H1, H2 and H3 as shown below. The concentrations of Standard 1-7 are 0.1, 0.2, 0.3, 0.4, 0.6, 0.8 and 1.0 µg/ 100 g (mL).

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Blank	Blank									
B	St1	St1	St1									
C	St2	St2	St2									
D	St3	St3	St3									
E	St4	St4	St4									
F	St5	St5	St5									
G	St6	St6	St6									
H	St7	St7	St7									

4) Add 100 µL of each prepared sample to the remaining microtiter wells.

5) Seal the wells on the strip with a sealing film and press the film to ensure that all wells are adequately sealed.

8.5 Incubate at 36℃ ± 1℃ for 44-48 h in an incubator, avoiding light.

8.6 Measurement

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.
2) Remove the sealing film diagonally and puncture the air bubbles on the surface of each well with a needle.
3) Measure the absorbance at 550 or 630 nm. 550nm is recommended.
Note: If the absorbance cannot be measured in time after incubation, keep the plate at 2-8°C for no more than 48 h.

9. Data analysis

9.1 Determination of validity of test results:

OD values of low concentration standards < OD values of high concentration standards

9.2 Select the optimally diluted sample to calculate the results:

For each sample, which is diluted to three different levels of concentration, select the one(s) whose OD value locates at the middle of the standard curve. In case two or more are in the middle, calculate the average result.
Use the 4-Parameter calculation formula in professional ELISA statistical analysis software to calculate the concentration of pantothenic acid in the samples. (Note: when multiple the dilution factors of samples, do NOT consider the 20X dilution during extraction.)

Note: The consumables required for the experiment must be sterile; waste must be disposed of after the experiment in accordance with the relevant regulations.

Note:

For infant and young children's complementary foods samples like baby rice cereal or baby noodles, sample preparation protocol for milk powder can also be applied. However, due to the high starch content of these products, a colloidal state is easily formed during the extraction process, which results in the incapability to apply membrane filtration directly after the extraction. In this case, the sample extract can be diluted first and then filtered to remove bacteria and debris. Use the filtrate for assay.
For the determination of folic acid in food that originally contains it, such as fruits, vegetables, meat, animal offal, certain enzymes are needed for preparation due to complexation with proteins. Use the appropriately prepared filtrate for the assay.

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

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