# NATIONAL STANDARD OF THE

# PEOPLE'S REPUBLIC OF CHINA



# GB 5009.211-2022

## National food safety standard - Determination of folates in food

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## Foreword

This Standard serves as a replacement of GB 5009.211-2014 National Food Safety Standard -

Determination of Folates in Food.

In comparison with GB 5009.211-2014, the main changes are as follows.

---the determination method with microplate is added;

---the requirements for precision are modified;

---Appendix A is modified.

National Food Safety Standard - Determination of Folates in

Food

## 1 Scope

This Standard specifies the methods of determining folates in food.

This Standard is applicable to the determination of folates in food.

## 2 Principle

Folates is an essential nutrient for the growth of Lactobacillus rhamnosus. Under certain

controlled conditions, inoculate the Lactobacillus rhamnosus bacterial solution into the medium

containing the specimen solution; after culturing for a period of time, determine the light

transmittance (or absorbance value). Within a certain determination range, in accordance with

the standard curve of folates content and light transmittance (or absorbance value), the folates

content in the specimen can be calculated.

## 3 Reagents and Materials

Unless it is otherwise specified, the reagents used in this Method are analytically pure; the water

is Grade-2 water specified in GB/T 6682.

**3.2.3 Sodium hydroxide ethanol solution (0.01 mol/L). weigh-take 0.4 g of sodium hydroxide;**

use 20% ethanol solution to dissolve and reach a constant volume of 1 L; mix it up.

**3.2.4 Sodium hydroxide solution (1 mol/L). weigh-take 40 g of sodium hydroxide; add water**

to dissolve and reach a constant volume of 1 L; mix it up.

**3.2.5 Hydrochloric acid soaking solution. measure-take 100 mL of hydrochloric acid**

(concentration. 36% ~ 38%); mix it up with 50 times of water.

**3.4 Reference Substance**

Folates reference substance (C19H19N7O6, CAS. 59-30-3). purity ? 97%, or reference substances

certified by the state and awarded with a reference substance certificate.

**5.1 Strains**

Lactobacillus rhamnosus (ATCC 7469) or equivalent strains.

**5.3 Preparation of Inoculum**

One day before the experiment, take 2 mL of the folates standard working solution and mix it

with 4 mL of the medium for folates determination, then, divide it into two test tubes.

### 6.2.1 Direct extraction method

When determining the content of folates added in the sample, the direct extraction method can

be adopted.

Accurately weigh-take 0.1 g ~ 2 g of solid specimen or 0.5 mL ~ 2 mL of liquid specimen, accurate to 0.001 g; transfer it into a conical flask. Add 80 mL of sodium hydroxide ethanol solution, with a stopper. Perform ultrasonic oscillation for 0.5 h ~ 4 h, until the specimen is completely dissolved or dispersed, then, transfer it into a 100 mL volumetric flask; use water to dilute to the scale.



### 6.2.2 Enzymatic extraction method

The enzymatic extraction method should be adopted for the naturally occurring folates in food

specimens, such as. cereals, potatoes, meat, eggs, dairy, fruits, vegetables, bacteria, algae, beans

and nuts, etc.

## 6.3 Dilution

In accordance with the folates content in the specimen, use water to appropriately dilute the specimen extract, so that the folates content in the specimen diluent is within the range of 0.2 ng/mL ~ 0.3 ng/mL.

### 6.4.1.1 Specimen and enzyme blank series tubes

Take three test tubes; respectively add 1.0 mL, 2.0 mL and 3.0 mL of specimen diluent (Vx); add water to 5.0 mL; mix it up. Take another three test tubes; adopt the same method to add enzyme blank solution. For each gradient, perform 2 parallels.

### 6.4.1.4 Inoculation and culture

After the series tubes used for determination are cooled down to room temperature, under the

conditions of aseptic operation, add 40 ?L of inoculum to each 10 mL of the medium for folates

determination; mix it up. Add 5 mL of the inoculated medium for folates determination to each

determination tube; mix it up. Place it in a constant-temperature incubator at 36 ?C ? 1 ?C to

culture for 20 h ~ 40 h. When the maximum turbidity is obtained, terminate the culture. Prepare

another standard 0 tube (containing 0.00 ng of folates) that is not inoculated and regard it as the

#### 6.4.1.5 Determination

Use a vortex oscillator to mix the cultured standard series tubes, specimens and enzyme blank

series tubes. Use a 1 cm cuvette, at 540 nm, adjust the light transmittance to 100% (or the

absorbance value is 0) with the 0 control tube that is not inoculated; successively determine the

light transmittance (or the absorbance value) of the standard series tubes, specimens and

enzyme blank series tubes. If the 0 control tube is turbid, it suggests that it may be contaminated

with bacteria, and the experiment needs to be re-performed.

#### 6.4.2.1 Specimen series tubes

Firstly, under aseptic conditions, use a sterile aqueous filter (0.22 ?m) to filter and sterilize the

specimen diluent in 6.3.Take three 1.5 mL sterile centrifuge tubes; respectively add 100 ?L,

## 200 ?L and 300 ?L of the specimen diluent; add sterile water to 500 ?L. For each gradient,

perform 2 parallels.

### 7.1 Standard Curve

Take the folates content of the standard series tubes as the x-coordinate; take the average value