# GB

NATIONAL STANDARD

OF THE PEOPLE'S REPUBLIC OF CHINA

# GB 5009.259-2016

## National food safety standard

## Determination of biotin in foods



**Issued on: August 31, 2016** **Implemented on: March 01, 2017**

**Issued by: National Health and Family Planning Commission of the PRC**

# Table of contents

# National food safety standard

# Determination of Biotin in foods

## 1 Scope

This standard specifies the method for determination of biotin in food.

This standard applies to the determination of biotin in food.

## 2 Principles

Biotin is a nutrient necessary for the growth of Lactobacillus plantarum. In the biotin determination medium, the growth of Lactobacillus plantarum is in linear relation with the biotin content in the sample to be determined; AND the content of the substance to be determined in the sample is calculated by comparing the light transmittance with the standard working curve.

## 3 Reagents and materials

Unless otherwise stated, the reagents used in this method are of analytical grade AND water is level II water as specified in GB/T 6682.

### 3.1 Reagents

**3.1.1** Anhydrous ethanol ($C_2H_6O$).

**3.1.2** Sodium hydroxide (NaOH).

**3.1.3** Hydrochloric acid (HCl).

**3.1.4** Citrate.

**3.1.5** α-amylase: ≥ 1.5 U/mg.

**3.1.6** Papain: ≥ 5 U/mg.

**3.1.7** Sulfuric acid ($H_2SO_4$).

### 3.2 Reagent preparation

**3.2.1** Ethanol solution (50%): MEASURE 500 mL of anhydrous ethanol; MIX it uniformly with 500 mL of water.

**3.2.2** Sodium hydroxide solution (0.5 mol/L): WEIGH 20 g of sodium hydroxide; DISSOLVE it into 1000 mL of water; MIX it uniformly.

**3.2.3** Sodium chloride solution (0.85%): WEIGH 8.5 g of sodium chloride; ADD water to dissolve and dilute it to 1000 mL; MIX it uniformly.

**3.2.4** Hydrochloric acid solution (1 mol/L): ABSORB 83 mL of hydrochloric acid; USE water to dilute it to 1000 mL; MIX it uniformly.

**3.2.5** Citrate buffer solution (pH 4.5): WEIGH 1.5 g of citric acid into a 100 mL beaker with a magnetic stirrer; ADD about 50 mL of distilled water to dissolve it; then ADD 12 mL of NaOH (1 mol/L); ADJUST the pH to 4.5 (with 0.1 mol/L HCl); TRANSFER the solution into a 100 mL volumetric flask; USE distilled water to make the volume reach to the mark. AND this butter solution can be preserved for 3 d at 2 °C ~ 8 °C.

**3.2.6** Protease-amylase solution: Respectively WEIGH 200 mg of papain and α-amylase; ADD 20 mL of water and GIND it to homogenate; CENTRIFUGE it at 3000 r/min for 5 min ~ 10 min; PREPARE it before use.

**3.2.7** Sulfuric acid solution (3%): MEASURE 30 mL of sulfuric acid; ADD it to 1000 mL of water; MIX it uniformly.

**3.3 Standard substance**

Biotin (d-Biotin or Vitamin H) standard substance ($C_{10}H_{16}N_2O_3S$): purity ≥ 99%.

**3.4 Standard solution preparation**

**3.4.1** Biotin standard stock solution (100 μg/mL): Accurately WEIGH 100 mg of biotin standard substance; USE ethanol solution (50%) to dissolve and transfer it into a 1000 mL volumetric flask; MAKE its volume reach to the mark. PRESERVE it in a brown bottle, which can be stored in a 2 °C ~ 4 °C refrigerator for 12 months.

**3.4.2** Biotin standard intermediate solution (1.0 μg/mL): Accurately PIPETTE 1.00 mL of biotin standard stock solution into a 100 mL brown volumetric flask; USE ethanol solution (50%) to dissolve it and MAKE its volume reach to the mark. PRESERVE it in a brown bottle, which can be stored in a 2 °C ~ 4 °C refrigerator for 6 months.

**3.4.3** Biotin standard working solution (10 ng/mL): Accurately PIPETTE 1.00 mL of biotin standard intermediate solution into a 100 mL volumetric flask; USE

machine; such samples as fruit and vegetable and semi-solid need to be mixed uniformly into homogenate; AND the liquid sample needs to be shaken before use to mix it, which is then preserved in a 4 °C refrigerator AND determined within 1 week.

**6.2 Sample extraction**

**6.2.1** Potato, meat, dairy, fresh fruits and vegetables, algae samples, eggs, beans, nuts, animal offal and other natural foods: Accurately WEIGH an appropriate amount of homogeneous sample (m) (containing about 0.2 μg ~ 0.5 μg biotin), accurate to 0.001 g; PLACE it into a 50 mL volumetric flask; ADD 30 mL of citric acid buffer solution; SHAKE it and MAKE it subjected to high pressure hydrolysis at 121 °C for 15 min. TAKE out the sample; immediately COOL it to room temperature; ADD 1 mL of protease-amylase solution; PLACE it into a 36 °C ± 1 °C constant temperature incubator for incubation and enzymolysis for 16 h ~ 20 h; HEAT it in the 95 °C water batch for 30 min; then rapidly COOL it to room temperature; TRANSFER it into a 100 mL volumetric flask; USE water to make the volume reach to the mark ($V_1$).

**6.2.2** Infant formula, cereals and other products (including raw and added biotin): Accurately WEIGH an appropriate amount of sample (m) (containing about 0.2 μg ~ 0.5 μg biotin), accurate to 0.001g; PLACE it into a 250 mL volumetric flask; ADD 100 mL of sulfuric acid solution; MAKE it subjected to hydrolysis at 121 °C for 30 min. After cooling, USE the sodium hydroxide solution to adjust pH to 4.5 ± 0.2; TRANSFER it into a 250 mL volumetric flask; USE water to make the volume reach to the mark; MIX it thoroughly. USE filter paper to filter it; DISCARD the first few milliliters; ABSORB 5 mL of the filtrate; ADD about 20 mL of water; USE sodium hydroxide solution to adjust the pH to 6.8 ± 0.2; TRANSFER it into a 100 mL volumetric flask; USE water to make the volume reach to the mark ($V_1$).

**6.2.3** Strengthened biotin beverages or vitamin premixes and other samples: as for the liquid beverage, ADD 5 mL ~ 10 mL of sample to a 100 mL volumetric flask; ADD 50 mL of water; MIX it uniformly; TRANSFER it into a 100 mL volumetric flask; USE water to make the volume reach to the mark ($V_1$); as for the vitamin premixes: Accurately WEIGH appropriate amount of sample (m), accurate to 0.001 g; PLACE it into a 500 mL volumetric flask; ADD about 300 mL of water; MIX it uniformly. ADJUST the pH to 8.0 ± 0.2; TRANSFER it into a 1000 mL volumetric flask; USE water to make the volume reach to the mark ($V_1$).

**6.3 Dilution**

# Appendix A

## Medium and reagent

### A.1 Lactobacillus agar medium

#### A.1.1 Component

TAKE 15.0 g of peptone milk, 5.0 g of yeast extract, 10.0 g of glucose, 100 mL of tomato juice, 2.0 g of potassium dihydrogen phosphate, and 1.0 g of polysorbate monooleate; ADD water to 1000 mL; ADJUST the pH to 6.8 ± 0.2 (25 °C ± 5 °C).

#### A.1.2 Preparation method

ADD 10.0 g of agar into A.1.1; HEAT to boil it to dissolve the agar; after mixing it uniformly, CONTAIN it into different test tubes, 10 mL for each tube. STERILIZE it at 121 °C under high pressure for 15 min; PREPARE for use.

### A.2 Lactobacillus broth medium

#### A.2.1 Ingredients

TAKE 15.0 g of peptone milk, 5.0 g of yeast extract, 10.0 g of glucose, 100 mL of tomato juice, 2.0 g of potassium dihydrogen phosphate, and 1.0 g of polysorbate monooleate; ADD water to 1000 mL; ADJUST the pH to 6.8 ± 0.2 (25 °C ± 5 °C).

#### A.2.2 Preparation method

HEAT the ingredients in A.2.1 to boil it; MIX it uniformly and CONTAIN it into different test tubes, 10 mL for each tube. STERILIZE it at 121 °C under high pressure for 15 min

### A.3 Biotin determination medium

#### A.3.1 Ingredients

TAKE 12.0 g of casein amino acid, 40.0 g of glucose, 20.0 g of sodium acetate, 0.2 g of L-cystine, 0.2 g of DL-tryptophane, 20.0 mg of adenine sulfate, 20.0 mg of guanine hydrochloride, 20.0 mg of uracil , 2.0 mg of thiamine hydrochloride, 2.0 mg of riboflavin, 2.0 mg of nicotinic acid, 2.0 mg of pantothenate, 4.0 mg of pyridoxine hydrochloride, 200.0 g of ρ-aminobenzoic acid, 1.0 g of dipotassium hydrogen phosphate, 1.0 g of magnesium sulfate,