



GB 5009.259-2016 National Food Safety Standard Determination of Biotin in Foods

GB 5009.259—2016 食品安全国家标准
食品中生物素的测定

NHFPC

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National Standard of the People's Republic of China

GB 5009.259-2016

National Food Safety Standard Determination of Biotin in Foods

食品安全国家标准

食品中生物素的测定

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Preface

This standard replaces GB 5413.19-2010 “Determination of free biotin in foods for infants and young children, milk and milk products”.

Compared with GB 5413.19-2010, the major changes of this standard are as follows:

- the name of the standard has been revised to “National Food Safety Standard Determination of Biotin in Foods”;
- the expression for preparing tubes for making the standard curve and test solution tubes has been revised;
- the expression of determination procedures has been revised;
- the processing procedures for food such as cereals and potatoes, meat, fresh fruits and vegetables, algae test samples, eggs, beans, nuts, animal organ meat and fortified biotin have been added;
- the description of generally used glassware in “Apparatus and Equipment” has been deleted.

National Food Safety Standard

Determination of Biotin in Foods

1 Scope

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

This standard applies to the determination of biotin in foods.

2 Principles

Biotin is a nutrient essential for the growth of *Lactobacillus plantarum*. In the culture medium for biotin determination, the growth of *Lactobacillus plantarum* bears a linear relation with the content of biotin in the test sample, and then the content of biotin in the test sample can be calculated based on the comparison of the light transmittance and the standard working curve.

3 Reagents and Materials

Unless otherwise specified, all the reagents used in this method are analytical reagents, and the water is the Grade 2 water 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 and mix it well with 500 mL of water.

3.2.2 Sodium hydroxide solution (0.5 mol/L): weigh 20 g of sodium hydroxide, dissolve it in 1000 mL of water, and mix well.

3.2.3 Sodium chloride solution (0.85%): weigh 8.5 g of sodium chloride, add water to dissolve and then dilute it to 1000 mL and mix well.

3.2.4 Hydrochloric acid solution (1 mol/L): pipette 83 mL of hydrochloric acid, and then dilute it to 1000 mL with water and mix well.

3.2.5 Citrate buffer solution (pH 4.5): weigh 1.5 g of citric acid into a 100-mL beaker with magnetic stirrer, add about 50 mL of distilled water to dissolve it and then add 12 mL of NaOH (1 mol/L) and adjust pH to 4.5 with 0.1 mol/L HCl; transfer the resulting solution to a 100-mL volumetric flask and then bring to volume with distilled water; this resulting buffer solution can be kept 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 grind it to homogenate and then centrifuge for 5 min~10 min at the speed of 3000 r/min; this solution shall be freshly prepared before use.

3.2.7 Sulfuric acid solution (3%): measure 30 mL of sulfuric acid, add it to 1000 mL of water and then mix it well.

3.3 Standard substances

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

3.4 Preparation of standard solution

3.4.1 Biotin standard stock solution (100 μ g/mL): accurately weigh 100 mg of biotin standard substance, dissolve it with ethanol solution (50%) and then transfer it into a 1000-mL volumetric flask and bring it to volume. This resulting solution can be kept in a brown flask inside the refrigerator for 12 months at 2 °C~4 °C.

3.4.2 Biotin standard intermediate solution (1.0 μ g/mL): accurately pipette 1.00 mL of biotin standard stock solution and place it in a 100-mL brown volumetric flask and then dilute it to volume with ethanol solution (50%) and mix it well; this resulting solution can be kept for 6 months in a bottle at 2 °C~4 °C in refrigerator.

3.4.3 Biotin standard working solution (10 ng/mL): accurately pipette 1.00 mL of biotin standard intermediate solution and place it in a 100-mL volumetric flask and then dilute it to volume with water and mix it well; this solution shall be freshly prepared before use.

3.4.4 Standard working solution (10 ng/mL): prepare the working solution with 2 concentrations. The higher concentration is 0.2 ng/mL and the lower concentration is 0.1 ng/mL. Pipette 5 mL of biotin standard working solution twice and dilute to 250 mL and 500 mL respectively with water.

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