



**NATIONAL STANDARD OF THE PEOPLE'S REPUBLIC  
OF CHINA**

**中华人民共和国国家标准**

**GB 5413.20-2013**

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**National Food Safety Standard  
Determination of Choline in Infant Food and  
Dairy Food**

**食品安全国家标准  
婴幼儿食品和乳品中胆碱的测定**

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People's Republic of China**



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## Foreword

This Standard replaces the GB/T 5413.20-1997 *Milk Powder and Formula Foods for Infant and Young Children-Determination of Choline*. Compared this standard with GB/T 5413.20-1997, main changes are as follows:

- The name of this Standard has been modified;
- The composition of the chromogenic agent used for the enzymatic reaction has been modified;
- The second method of Reinecke salt spectrophotometric method has been added.

**National Food Safety Standard**  
**Determination of Choline in Infant Food and Dairy Food**

**1 Scope**

This Standard has stipulated the determination method for the choline in the infant food and dairy food.

This Standard is applicable to the determination for the choline in the infant food and dairy food.

**The First method      Enzyme colorimetric method**

**2 Principle**

The choline in a sample turns to Free State after acid hydrolysis, and then the colored matter is created by the reaction with chromogenic agent after the choline is disposed by enzymatic oxidation. The different shade of the color is proportional to the content of choline in a certain concentration range.

**3 Reagents and materials**

Note: Unless otherwise noted, the reagent used in this method is analytically pure and the water is grade III water specified in GB/T 6682.

**3.1 Reagents**

- 3.1.1** Tris (trihydroxymethyl aminomethane)  $[(\text{CH}_2\text{OH})_3\text{CNH}_2]$ .
- 3.1.2** Phenol ( $\text{C}_6\text{H}_5\text{OH}$ ).
- 3.1.3** Concentrated hydrochloric acid (HCL).
- 3.1.4** Sodium hydroxide (NaOH).
- 3.1.5** Choline oxidase: Placed at  $-20^\circ\text{C}$  for preservation.
- 3.1.6** Peroxidase: Placed at  $2^\circ\text{C}$ - $8^\circ\text{C}$  for preservation.
- 3.1.7** 4-amino antipyrine ( $\text{C}_{11}\text{H}_{13}\text{N}_3\text{O}$ ).
- 3.1.8** Phospholipase D: Placed at  $-20^\circ\text{C}$  for preservation.

**3.2 Reagent preparation**



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