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

SN/T 5216-2020

Commercial kit detection method Nisin Method 1

商品化试剂盒检测方法 乳酸链球菌素 方法一

(English Translation)

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Foreword

This standard was drafted in accordance with the rules given in GB/T 1.1-2009.

This standard was proposed and managed by the General Administration of Customs of the People's Republic of China.

Drafting organizations of this standard. Urumqi Customs of the People's Republic of China, Beijing Qinbang Biotechnology Co., Ltd.

1 Scope

This standard specifies the enzyme-linked immunoassay method for nisin in food.

This standard is applicable to the detection of the content of nisin A and nisin Z in foods

such as milk-containing beverages and meat products.

2 Normative references

The following documents are indispensable for the application of this document. For dated reference documents, only the dated version applies to this article. For undated reference documents, the latest version (including all amendments) is applicable to this document.

GB/T 6682 Analytical laboratory water specifications and test methods
SN/T 2775 Evaluation Method of Commercial Food Testing Kit

3 Terms and definitions

The following terms and definitions apply to this document.

Nisin

4 Method summary

The nisin in the sample and the antigen pre-coated on the microwell strip in the kit compete for the nisin antibody, add enzyme label two. After anti-immunization, use the substrate color developing solution to develop the color. The sample absorbance value is negatively correlated with the content of nisin, which is compared with the standard curve and then multiplied by the corresponding dilution factor can be used to obtain the content of nisin in the sample.

5 Materials and reagents

Unless otherwise specified, all reagents are analytically pure, and the water is the first grade water specified in GB/T 6682.

5.1

Nisin test kit. Refer to SN/T 2775 for kit evaluation, and refer to Appendix A for specific evaluation results. Kit composition as follows.

- a) 96-well microtiter plate. 12 strips x 8 wells (coated with coupled antigen);
- b) Nisin standard. 0 mg/L, 0.1 mg/L, 0.5 mg/L, 2.5 mg/L, 12.5 mg/L, 62.5 mg/L;
- c) High-concentration standard. 100 mg/L;
- d) Enzyme marker concentrate;
- e) Antibody working solution;
- f) Concentrated cleaning solution;

g) Sample extraction solution;

h) Chromogenic solution. Substrate A, Substrate B;

i) Stop solution.

The kit should be stored at 4 °C ~ 8 °C protected from light, and the dissolved enzyme label should be frozen at -15 °C.

5.2

Hydrochloric acid.

5.3

Sodium hydroxide.

5.4

n-hexane.

6 Instruments

6.1

Balance. the range is 100 g, and the sensitivity is 0.01 g.

6.2

Microplate reader. wavelength 450 nm.

6.3

Vortex apparatus.

6.4

Centrifuge.

6.5

Hybrid oscillator.

6.6

Single channel pipette. 20 µL~200 µL, 100 µL~1 000 µL, 500 µL~5 000 µL.

6.7

Multi-channel pipette. 30 µL ~ 300 µL.

6.8

Polystyrene centrifuge tube. 2 mL, 10 mL, 50 mL.

6.9

Mortar or crushing device.

7 Preparation and extraction of samples**7.1**

Milk and beverage liquid samples. Dilute the sample 10 times with the sample extract and take 50 μ L for analysis. The sample dilution factor is 10.

7.2

Solid samples of dairy products and meat products. Weigh 25 g (accurate to 0.01 g) samples, and use a mortar or crushing device to make the samples in powder form or granular form with a diameter of less than 0.3 mm, weigh 1 g (accurate to 0.01 g) of the prepared sample in powder form to a 50 mL centrifuge tube. Add 5 mL of sample extract, vortex and mix for 5 min, centrifuge at 3,000 r/min for 5 min, then stand for 10 min, and extract the supernatant. Put 1 mL into the centrifuge tube (be careful not to suck the oil on the surface and the sediment on the bottom layer), and take 50 μ L for analysis. The sample dilution factor is 5.

8.1 Operating conditions

All operations should be carried out at room temperature (20 °C~25 °C), and the temperature of all reagents in the nisin kit should be raised to can be used after room temperature.

8.2 Washing conditions**8.2.1**

The number of manual washing is 4 times, and 250 μ L/well of washing working solution is injected each time.

8.2.2

The automatic plate washing is scheduled for 4 times.

8.3 Determination conditions of microplate reader

The measurement wavelength of the microplate reader is 450 nm.

8.4 Measurement procedure**8.4.1**

Insert the microwell strip required for the measurement into the microwell rack, and mark the position of the standard solution and sample on the microwell rack.