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NATIONAL STANDARD
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GB 4789.35-2016

**National food safety standard – Microbiological
examination of food – Examination of lactic acid bacteria**

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Table of contents

Foreword.....	3
1 Scope	4
2 Terms and definitions	4
3 Equipment and materials.....	4
4 Medium and reagents.....	4
5 Testing procedures.....	5
6 Operating procedures.....	6
7 Results and reports	10
8 Identification of lactic acid bacteria (optional)	10
Appendix A Medium and reagents	12

Foreword

This standard replaces GB 4789.35-2010 “National food safety standard – Microbiological examination of food – Examination of lactic acid bacteria” AND SN/T 1941.1-2007 “Detection of lactic acid bacteria in food for import and export – Part 1: isolation and enumeration method”.

As compared with GB 4789.35-2010, the main changes of this standard are as follows:

- ADD the selection and result description of the enumeration and culture conditions for total number of lactic acid bacteria;
- MODIFY the modified MRS medium components;
- MODIFY the plate count inoculation method and inoculation amount.

National food safety standard – Microbiological examination of food – Examination of lactic acid bacteria

1 Scope

This standard specifies the testing method of the lactic acid bacteria in the food containing lactic acid bacteria.

This standard is applicable to the testing of lactic acid bacteria in foods containing active lactic acid bacteria.

2 Terms and definitions

2.1

Lactic acid bacteria

It is a general term of the bacteria for which the fermentable sugars mainly produce large amounts of lactic acid. The lactic acid bacteria in this standard are mainly *Lactobacillus*, *Bifidobacterium* and *Streptococcus*.

3 Equipment and materials

In addition to routine sterilization and culture equipment for microbiological laboratories, other equipment and materials are as follows:

3.1 Constant temperature incubator: $36\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$.

3.2 Refrigerator: $2\text{ }^{\circ}\text{C} \sim 5\text{ }^{\circ}\text{C}$.

3.3 Homogenizer and sterile homogeneous bag, homogeneous cup or sterilized mortar.

3.4 Balance: sensitivity of 0.01 g.

3.5 Sterile test tube: 18 mm × 180 mm, 15 mm × 100 mm.

3.6 Sterile pipettes: 1 mL (with 0.01 mL scale), 10 mL (with 0.1 mL scale) OR micro pipette and suction head.

3.7 Sterile conical flask: 500 mL, 250 mL.

4 Medium and reagents

4.1 Normal saline: SEE A.1.

In accordance with the estimation of the content of the Bifidobacterium contained in the sample under test, SELECT 2 ~ 3 continuous appropriate dilutions. For each dilution, ABSORB 1 mL of sample homogenate into a sterile plate; PREPARE two places for each dilution. After transferring the diluent into the plate, POUR 15 mL of Li-Mupirocin and Cysteine Hydrochloride modified MRS agar culture medium which are cooled to 48 °C into each plate; ROTATE the plate to mix it uniformly. CULTURE it in the 36 °C ± 1 °C anaerobic environment for 72 h ± 2 h; after culture, COUNT the number of colonies on the plate. The duration from sample dilution to the plate pouring shall be finished within 15 min.

6.2.3.3 Count of Streptococcus

In accordance with the estimation of the viable cell number of Streptococcus, SELECT 2 ~ 3 continuous appropriate dilutions; for each dilution, ABSORB 1 mL of sample homogenate into a sterile plate; PREPARE two plates for each dilution. After transferring the diluent into the plate, POUR about 15 mL of MC culture medium which is cooled to 48 °C into the plate; ROTATE the plate to mix it uniformly. CULTURE it in the 36 °C ± 1 °C aerobic environment for 72 h ± 2 h; after culture, COUNT the number. The colonies of the Streptococcus on the MC agar plate are characterized by: colony size is medium to small, the colony is red with neat and smooth edge, the diameter is 2 mm ± 1 mm, AND the colony back is pink. The duration from sample dilution to the plate pouring shall be finished within 15 min.

6.2.3.4 Count of Lactobacillus

In accordance with the estimation of the viable cell number of the sample under test, SELECT 2 ~ 3 continuous appropriate dilutions; for each dilution, ABSORB 1 mL of sample homogenate into a sterile plate; PREPARE two plates for each dilution. After transferring the diluent into the plate, POUR about 15 mL of MC agar culture medium which is cooled to 48 °C into the plate; ROTATE the plate to mix it uniformly. CULTURE it in the 36 °C ± 1 °C anaerobic environment for 72 h ± 2 h. The duration from sample dilution to the plate pouring shall be finished within 15 min.

6.3 Count of colony

Note: it may make observation using naked eyes, AND if necessary USE a magnifying glass or colony counter; RECORD the dilution factor and the corresponding number of colonies. The colony count is expressed in colony-forming units (CFU).

6.3.1 SELECT the plate wherein the number of colonies is between 30 CFU ~ 300 CFU AND there is no colony spreading growth to count the total number of colonies. RECORD the actual number of colonies for the plate having less than 30 CFU, and the plate having more than 300 CFU can be recorded as countless. The number of colonies per dilution shall use the average of two plates.

Appendix A

Medium and reagents

A.1 Physiological saline

A.1.1 Ingredients

NaCl: 8.5 g

A.1.2 Methodology

ADD the above ingredient into 1000 mL distilled water; HEAT to dissolve it; SEPARATE it and MAKE it be autoclaved at 121 ° C for 15 min ~ 20 min.

A.2 MRS medium

A.2.1 Ingredients

Peptone: 10.0 g

Beef powder: 5.0 g

Yeast powder: 4.0 g

Glucose: 20.0 g

Tween 80: 1.0 mL

K₂HPO₄ • 7H₂O: 2.0 g

Sodium acetate • 3H₂O: 5.0 g

Triodium citrate: 2.0 g

MgSO₄ • 7H₂O: 0.2 g

MnSO₄ • 4H₂O: 0.05 g

Agar powder: 15.0 g

A.2.2 Methodology

ADD the above ingredient into 1000 mL distilled water; HEAT to dissolve it; ADJUST the pH to 6.2 ± 0.2; SEPARATE it and MAKE it be autoclaved at 121 ° C for 15 min ~ 20 min.

A.3 Li-Mupirocin and Cysteine Hydrochloride modified MRS medium

Peptone: 5.0 g

Yeast extract: 5.0 g

Tween 80: 0.5 mL

Agar: 1.5 g

1.6% bromocresol purple alcohol solution: 1.4 mL

Distilled water: 1000 mL

A.5.2 Methodology

ADD the required carbohydrate by 0.5%; CONTAIN it into small test tubes; MAKE it be autoclaved at 121 ° C for 15 min ~ 20 min.

A.6 Aescinate medium

A.6.1 Ingredients

Peptone: 5.0 g

Dipotassium phosphate: 1.0 g

Aescinate: 3.0 g

Citric acid: 0.5 g

1.6% bromocresol purple alcohol solution: 1.4 mL

Distilled water: 100 mL

A.6.2 Methodology

ADD the above ingredients into distilled water; HEAT to dissolve it; MAKE it be autoclaved at 121 ° C for 15 min ~ 20 min.

A.7 Gram stain

A.7.1 Crystal violet dyeing solution

A.7.1.1 Ingredients

Crystal violet: 1.0 g

95% ethanol: 20 mL

1% ammonium oxalate aqueous solution: 80 mL

A.7.1.2 Methodology

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