



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

GB 4789.43-2016

**National food safety standard - Food microbiological
examination - Microbial source enzyme preparation
antibacterial activity determination**

**食品安全国家标准 食品微生物学检验 微生物源酶制剂抗菌活性的测定
(English Translation)**

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1 Scope

This Standard specifies the method for the determination of antibacterial activity of microbial source enzyme preparation.

This Standard is applicable to the determination of antibacterial activity of enzyme preparation produced by microorganism.

2 Equipment and material

In addition to microbial laboratory routine sterilization and training equipment, other equipment and materials are as follows.

2.1

biological safety cabinet.

2.2

refrigerator. 2°C ~ 5°C.

2.3

constant temperature incubator. 36°C ± 1°C.

2.4

constant temperature water bath. 46°C ± 1°C.

2.5

balance. resolution of 0.1 g.

2.6

oscillator.

2.7

sterile pipettes. 1 mL (with 0.01 mL scale), 10 mL (with 0.1 mL scale) or micro pipette and suction head.

2.8

sterile Petri dish. 90mm in diameter.

2.9

sterile Erlenmeyer flask. capacity of 250 mL, 500 mL.

2.10

pH meter or precision pH test paper.

2.11

sterile paper. see A.8.

2.12

sterile tweezers.

6.2.1 Dilution of bacteria suspension stock solution

Respectively take the bacteria suspension stock solution prepared in 6.1 to carry out ten times gradient dilution in order. *Bacillus cereus* and *Bacillus circulans* shall be respectively prepared to 10^{-4} dilution solution.

Staphylococcus aureus, *Escherichia coli*, *Streptococcus pyogenes*, *Serratia marcescens* shall be respectively prepared to 10^{-5} dilution solution.

6.2.2 Culture count

Respectively pipet 1 mL of each bacteria dilution solution prepared in 6.2.1 into sterile plate. Each dilution shall be done in two parallel. Use sterile saline for blank control. Pour 15mL ~ 20mL of plate count agar that have been cooled to around 46°C (can be placed at a $46^{\circ}\text{C} \pm 1^{\circ}\text{C}$ constant temperature water bath box for insulation) into the plate. Rotate the plate to mix evenly.

After agar solidification, turn over the plate. Cultivate at $36^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 24h then count. Use when the bacterial concentration of each suspension stock solution is more than 10^6 CFU/mL.

6.3 Preparation of inspection plate

Pour 15 mL of TSA medium that is melted and cooled to $46^{\circ}\text{C} \pm 1^{\circ}\text{C}$ into sterile petri dish. Make it TSA plate after it is solidified, for use. Respectively use TSA medium that is cooled to $46^{\circ}\text{C} \pm 1^{\circ}\text{C}$ to dilute each culture after 6.1 second subculture according to a 1.10 ratio (streptococcus pyogenes ATCC

12344 shall be diluted according to 1.20). After well mixing, respectively take

10 mL into the TSA plate that is well prepared and insulated at $46^{\circ}\text{C} \pm 1^{\circ}\text{C}$.

Gently shake the plate to make bacteria solution evenly paved. Make it inspection plate after solidification, for use.

NOTE To prevent bacterial culture medium is solidified when it immediately meets TSA and cause uneven bacteria suspension solution pavement, it shall place the TSA plate at

$46^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 10 min insulation before bacteria suspension solution pavement.

6.4 Preparation of enzyme preparation paper

Accurately weigh (pipet) 1.0 g (mL) of enzyme preparation into 9 mL of sterile saline. After even mixing, prepare it to 10% enzyme preparation solution.

Place the sterile paper in a sterile plate. Slowly add 100 μL of 10% enzyme preparation solution on each piece of paper to make it completely absorbed.

Prepare 12 pieces of paper for each enzyme preparation sample.

A.4.1 Compositions

HCl 9 mL

Distilled water 991 mL

A.4.2 Preparation method

Pipet 9 mL of concentrated hydrochloric acid (mass concentration is 36%, density is 1.17 g/m³). Use sterile distilled water to dilute to 1000 mL. Use 0.22

μm microporous membrane to filter and sterilize for use.

A.5.1 Compositions

Sodium chloride 8.5 g

Distilled water 1000 mL

A.5.2 Preparation method

Pipet 8.5 g of Sodium chloride and dissolve into 1000 mL of distilled water.

Sterilize at 121°C for 15 min.

A.6 50.0 µg/mL ciprofloxacin (CIP)

A.6.1 Compositions

1000 µg/mL ciprofloxacin 0.5 mL

Sterile saline 9.5 mL

A.6.2 Preparation method

Weigh a certain mass of ciprofloxacin according to the product instruction (percentage, water content or potency). Use an appropriate amount of 0.1 mol/L HCl to dissolve to make its concentration 1000 µg/mL. Under aseptic conditions, use 0.22 µm microporous membrane to filter and pipet 0.5 mL.

Use sterile saline to dilute to 10 mL. Mix well.

A.7 5.0 µg/tablet ciprofloxacin paper (CIP paper)

A.7.1 Compositions

50.0 µg/mL ciprofloxacin 100 µL

Sterile paper 1 piece

A.7.2 Preparation method

Pipet 100 µL of 50.0 µg/mL ciprofloxacin solution. Slowly and evenly drop onto sterile paper to make 5.0 µg/tablet ciprofloxacin paper.

A.8 Sterile paper

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