



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

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**National food safety standard - Bacterial reversion mutation
test**

食品安全国家标准 细菌回复突变试验

(English Translation)

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1 Scope

Bacterial reverse mutation test includes *Salmonella typhimurium* reversion mutation test and *Escherichia coli* reversion mutation test. This Standard specifies the basic technical requirements for *Salmonella typhimurium* reversion mutation test. When selecting *Escherichia coli* for bacterial reversion mutation test, relevant literature shall be referred to.

This Standard is applicable to the evaluation of the mutagenic effect of test substances.

2.1 Bacterial reversion mutation test

An in vitro test for detecting gene mutations using auxotrophic mutant strains as indicator organisms. Commonly used strains include histidine auxotroph *Salmonella typhimurium* and tryptophan auxotroph *Escherichia coli*.

2.2 Base substitution gene mutation

A certain base on the DNA polynucleotide chain is replaced by another base, causing abnormalities in the DNA base sequence.

2.3 Frameshift gene mutation

When one or several bases (except 3 and multiples of 3) are inserted or deleted in the DNA base sequence, according to the rules of continuous reading of the triplet code, all the codon composition after this site will be changed, and the polypeptide chain that guides the

synthesis will also be completely changed.

3 Test purpose and principle

Detect the genetic mutation effect of the test substance on microorganisms (bacteria) and predict its genotoxicity and potential carcinogenic effects.

The bacterial reversion mutation test uses *Salmonella typhimurium* and *Escherichia coli* to detect point mutations, involving the replacement, insertion or deletion of one or several base pairs of DNA, as shown in Appendix A. The test strains of *Salmonella typhimurium* and *Escherichia coli* are histidine-deficient mutants and tryptophan-

80 °C, prepare plates at a rate of 25 mL per plate (relative to a 90 mm plate). After condensation and solidification, place the plates upside down in a 37 °C incubator for 24 h for use.

4.2.3.2 Phosphate stock solution (Vogel-Bonner minimal medium E, 50 times)

Sodium ammonium hydrogen phosphate ($\text{NaNH}_4\text{HPO}_4 \cdot 4\text{H}_2\text{O}$) 17.5 g

Citric acid ($\text{C}_6\text{H}_8\text{O}_7 \cdot \text{H}_2\text{O}$) 10.0 g

Dipotassium hydrogen phosphate (K_2HPO_4) 50.0 g

Magnesium sulfate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$) 1.0 g

Add distilled water to 100 mL to dissolve, and sterilize at 0.103 MPa for 20 minutes.

Note: After the other reagents are completely dissolved, slowly put the magnesium sulfate into it to continue dissolving, otherwise it will easily precipitate.

4.2.3.3 40% glucose solution

Add distilled water to 40.0 g of glucose to 100 mL, and sterilize at 0.055 MPa for 20 minutes.

4.2.3.4 1.5% agar medium

Add 6.0 g of agar powder into a 400 mL conical flask; add distilled water to 400 mL; after melting, sterilize at 0.103 MPa for 20 min.

4.2.4 Top culture medium

Heat to melt the top agar, add 10 mL of histidine-biotin solution (0.5 mmol/L) to every 100 mL of top agar. Mix well; divide into 4 flasks; sterilize at 0.103 MPa for 20 min.

Melt and divide into small test tubes, 2 mL per tube; keep warm in a 45 °C water bath.

Prepare top agar and histidine-biotin solution (0.5 mmol/L) as follows.

4.2.4.1 Top agar

Add distilled water to 3.0 g of agar powder and 2.5 g of sodium chloride to 500 mL; sterilize at 0.103 MPa for 20 min.

4.2.4.2 Histidine-biotin solution (0.5 mmol/L) (for mutagenesis test)

Add 250 mL of distilled water to 30.5 mg of D-biotin (relative molecular mass 244) and 19.4 mg of L-histidine (relative molecular mass 155); sterilize at 0.103 MPa for 20 min.

4.2.5 Special reagents and culture media

4.2.5.1 0.8% ampicillin solution (for strain identification, prepared aseptically)

Use sodium hydroxide solution (0.02 mol/L) to dilute 40 mg of ampicillin to 5 mL; store it in a refrigerator at 4 °C for later use.

4.2.5.2 0.1% crystal violet solution (for strain identification)

Dissolve 100 mg of crystal violet in sterile water to 100 mL.

4.2.5.3 L-histidine solution and D-biotin solution (0.5 mmol/L) (for strain identification)

Respectively dissolve 0.404 3 g of L-histidine and 12.2 mg of D-biotin in distilled water to 100 mL; sterilize at 0.103 MPa for 20 min; store in a 4 °C refrigerator for later use.

4.2.5.4 0.8% tetracycline solution (for tetracycline resistance test and ampicillin- tetracycline plate)

Use hydrochloric acid buffer (0.02 mol/L) to dilute 40 mg of tetracycline to 5 mL; store in a 4 °C refrigerator.

4.2.5.5 Ampicillin plate (used as the master plate for TA97, TA98, and TA100 strains) and ampicillin-tetracycline plate (used as the master plate for TA102 strain)

Each 1 000 mL consists of the following ingredients:

Bottom culture medium 980 mL

Histidine aqueous solution (0.404 3 g/100 mL) 10 mL

Biotin (0.5 mmol/L) 6 mL

0.8% ampicillin solution 3.15 mL

0.8% tetracycline solution 0.25 mL

Tetracycline is added only when using tetracycline-resistant TA102. Each ingredient has

been individually sterilized or prepared aseptically.

4.2.5.6 Histidine-biotin plate (histidine needs to be used for testing)

Each 1 000 mL consists of the following ingredients:

Bottom culture medium 984 mL

Histidine aqueous solution (0.404 3 g/100 mL) 10 mL

Biotin (0.5 mmol/L) 6 mL

Each ingredient has been sterilized individually.

oral gavage at a dose of 80 mg/kg for 3 consecutive days without fasting. Kill the animals by decapitation after 16 h of fasting. Other operations are the same as PCB induction.

After killing the animals, remove the liver, weigh, and continuously flush with fresh ice-cold potassium chloride solution (0.15 mol/L) several times to remove hemoglobin that inhibits microsomal enzyme activity. Add 3 mL of potassium chloride solution (0.1 mol/L) per gram of liver (wet weight); move it together with the beaker into an ice bath;

use sterile scissors to cut the liver into pieces; place in a glass homogenizer (less than 4 000 r/min, 1 min ~ 2 min) or tissue homogenizer (less than 20 000 r/min, 1 min) to make liver homogenate. The above operations require attention to sterility and local cold environment.

Centrifuge the prepared liver homogenate in a low-temperature (0 °C ~ 4 °C) high- speed centrifuge at 9 000g for 10 minutes. Aspirate the supernatant into the S9 component and distribute it into sterile freezing tubes or ampoules. Each ampoule is about 2 mL. Quick freeze in liquid nitrogen or dry ice and then store at -80 °C.

After the S9 component is prepared, subject it to a sterility inspection and measure the protein content (Lowry method). The protein content per milliliter should not exceed

40 mg. After qualifying the biological activity by indirect carcinogen (mutagen) identification, store it at a deep low temperature or freeze-dry, and the shelf life shall not exceed 1 year.

4.3.3 Preparation of 10% S9 mixture

The 10% S9 mixture is generally composed of S9 components and cofactors in a ratio of 1:9. The concentration can also be prepared to 30% (different test substances require different S9 concentrations). Freshly prepare aseptically or filter for sterilization before use. 10 mL of 10% S9 mixture is prepared as follows:

Phosphate buffer 6.0 mL

Magnesium potassium solution 0.4 mL