

ICS 13.300
CCS A80



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

GB/T 21786-2025

Replacing GB/T 21786-2008

Chemicals - Test method of bacterial reverse mutation

化学品 细菌回复突变试验方法

Issued on: August 29, 2025

Implemented on: December 1, 2025

**Issued by: State Administration for Market Regulation;
Standardization Administration of China**

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Foreword

This document complies with the provisions of GB/T 1.1-2020 "Standardization Work Guidelines Part

1. Structure and Drafting Rules of Standardization Documents". Drafting. This document supersedes GB/T 21786-2008 "Test Method for Bacterial Reversion Mutation of Chemicals" and is consistent with GB/T 21786-2008. Aside from structural adjustments and editorial changes, the main technical changes are as follows:

- a) The content for "bacteria" has been changed (see 5.1.1.4, 4.1.1.4 in the 2008 version);
- b) The content regarding "dosage" has been modified (see 5.2.2.1, 4.2.2.1 in the 2008 version);
- c) The content of "Comparison" has been changed (see 5.2.3.3, 4.2.3.3 in the 2008 version);
- d) The content of "Test Procedure" has been changed (see 5.3.1, 5.3.2, 4.3.1.2 in the 2008 version). Please note that some content in this document may involve patents. The issuing organization of this document assumes no responsibility for identifying patents. This document was proposed and is under the jurisdiction of the National Technical Committee on Standardization of Hazardous Chemicals Management (SAC/TC251). This document was drafted by: the National Institute for Occupational Health and Poison Control, Chinese Center for Disease Control and Prevention; and the National Institute for Environmental Health and Related Product Safety, Chinese Center for Disease Control and Prevention. Kang-related product safety institute, Pingdingshan Huixinyuan Biotechnology

Co., Ltd. The main drafters of this document are. Shen Meili, Dai Yufei, Duan Huawei, Chen Yuanyuan, Deng Fuchang, Shi Ying, Zhang Shaoping, Liu Shuai, and Shi Dongdong. The release history of this document and the document it replaces is as follows:

---First published in.2008 as GB/T 21786-2008;

---This is the first revision.

The *Escherichiacoli* strain was used to detect gene mutations, which involve substitutions, insertions, or deletions of one or more base pairs in the DNA. The principle of the experiment is to detect the ability of a test strain to restore its ability to synthesize essential amino acids due to a reversion mutation caused by the test substance. Reversion mutations occur... Bacteria can grow in the absence of certain essential amino acids, while parent strains cannot. Therefore, the number of colonies formed can be used to determine the presence of a pathogen. To determine whether the test substance is a mutagen. Gene mutations are a key factor leading to many hereditary diseases, and there is ample evidence that mutations in somatic oncogenes and tumor suppressor genes are a contributing factor. It is associated with tumor formation in humans and laboratory animals. The bacterial reverse mutation assay has advantages such as being rapid, economical, and relatively simple to operate. [Assay] The strain also possesses certain characteristics that make it more sensitive to mutation detection, such as the presence of responsive DNA sequences at reversion mutation sites, and the bacteria's responsiveness to large... Increased permeability of molecular substances, and enhanced defects in the DNA repair system or enhanced error-prone DNA repair processes. The specificity of the test strain can be... The types of mutations induced by genotoxic substances provide valuable research information. Bacterial reversion mutations containing various chemical structural substances have now been constructed. A database of test results provides access to relevant data. Furthermore, comprehensive testing methods have been established for chemicals with different physicochemical properties. The law includes volatile compounds. The bacterial reverse mutation assay uses prokaryotic cells, which are similar to mammalian cells in terms of absorption, metabolism, chromosome structure, and DNA repair processes. Significant differences were observed in mammalian cells. This experiment is an in vitro assay; exogenous metabolic activation systems can be added, but exogenous metabolic activation systems... The in vivo metabolic conditions cannot be fully simulated. Therefore, the results of this experiment cannot provide direct evidence for the mutagenic and carcinogenic effects of the test substance on mammals. Receive the evidence. Bacterial reverse mutation assays can be used for preliminary screening of genotoxicity, and are particularly suitable for detecting point mutations induced by the test substance. Numerous studies have shown that Data shows that many chemicals that tested positive in this test also showed mutagenicity in other tests, but some mutagens also showed mutagenicity in this test. It was not detected in the test. This limitation may be related to the specific nature of the detection endpoint, differences in metabolic activation, or differences in bioavailability. In addition, factors that improve the sensitivity of this test

may overestimate the mutagenic activity of the test substance. Bacterial reverse mutation assays are not suitable for evaluating certain classes of chemicals, such as compounds with strong bactericidal activity (e.g., some antibiotics). Compounds that specifically interfere with the mammalian cell replication system (such as certain topoisomerase inhibitors and certain nucleoside analogs). These test substances may be more suitable for mammalian cell mutagenicity assays. Although many of the compounds that tested positive in this study are mammalian carcinogens, the correlation is not absolute, but rather depends on the specific compound. The type of chemical is relevant. Some carcinogens induce cancer through non-genotoxic mechanisms or mechanisms lacking the tested strain, therefore they cannot be used to determine the specific type of carcinogen. It was detected during the test. Chemical Bacterial Reversion Mutation Test Method

1 Scope

GB/T 21786-2025 is the Chinese national standard on chemicals - test method of bacterial reverse mutation, in the field of environment and health protection, safety. The /T suffix marks it as a recommended standard: it is not compulsory by itself, but it becomes binding as soon as a contract, a tender or a customer specification calls it up - which in practice is how most foreign buyers meet it. It was issued on 29 August 2025 by the State Administration for Market Regulation; Standardization Administration of China, and has been in force since 1 December 2025. Classification: ICS 13.300, CCS A80. This page is published from the official record of the standard held by the Chinese standards administration: the identification, the dates, the classification and the issuing body are taken from there. The clause text, the tables and the numeric limits are in the document itself, which is delivered complete in English translation.

This document outlines the basic principles of bacterial reverse mutation assays, specifying the experimental methods, data collection, and reporting procedures. This document applies to the testing of the mutagenicity of chemicals (excluding those with bactericidal effects).

2 Normative references

This document has no normative references.

3 Terms and Definitions

The following terms and definitions apply to this document.

3.1 reverse mutation test An in vitro assay for detecting gene mutations using auxotrophic mutant strains as indicator organisms. (Escherichiacoli).

4 Basic Principles of the Experiment

4.1 Bacterial suspensions were mixed with the test substance under conditions with and without the addition of an exogenous metabolic activation system. The mixture was then incorporated into the agar plate method. In the pre-culture method, the bacterial suspension is first mixed thoroughly with the top agar plate, and then quickly poured onto the bottom agar plate. The test samples were pre-cultured, then thoroughly mixed with the top agar, and quickly poured onto the bottom agar plates. The plates were then incubated for 2-3 days. Then, the number of revertant colonies was counted and compared with the number of spontaneous revertant colonies in the solvent control group.

4.2 There are several methods for bacterial reverse mutation assays, the most common of which are the plate incorporation method, the pre-culture method, the wave method, and the suspension culture method. In addition, there are improved methods for detecting gases or vapors.

4.3 This document primarily describes the plate incorporation method and the pre-culture method. These two methods differ depending on whether a metabolic activation system is added or not. All conditions can be met. Pre-culturing is suitable for some chemicals, including short-chain aliphatic nitrosamines, divalent metals, aldehydes, azo dyes, and diazo compounds. Compounds, pyrrolizidine alkaloids, allyl compounds, and nitro compounds. Certain classes of chemicals are processed using standard methods (e.g., plate tests). Incorporation and pre-culture methods sometimes fail to detect positive results. Alternative methods should be used for these special samples. (Regarding azo dyes...) For the detection of special samples such as diazo compounds, gases, volatile chemicals, and glycosides, alternative methods have been reported in the literature. Regarding standards... Any deviation from the methodology should be based on scientific evidence.

5.1.1 Bacteria

5.1.1.1 Fresh bacterial suspensions should be cultured to the late logarithmic growth phase or early stationary phase (concentration approximately 10^9 cells/mL). Stable culture should not be used. The bacterial suspension at the late stage. The bacterial suspension used in the experiment should contain a high concentration of live bacteria. The concentration of live bacteria can be determined by the historical data of the bacterial growth curve. The results were determined by comparison data or by plate counting at each test.

5.1.1.2 The recommended culture temperature is 37°C.

5.1.1.3 At least five strains should be used. Recommended strain combinations are as follows:

- a) *Salmonella typhimurium* TA1535;
- b) *Salmonella typhimurium* TA1537, TA97, or TA97a;