CHEMLINKED
by REACH24H Consulting Group



Bacterial Reverse Mutation Test

细菌回复突变试验方法

National Medical Products Administration

Release Date: May 29, 2026
Implementation Date: Mar 1, 2027

Translated by ChemLinked

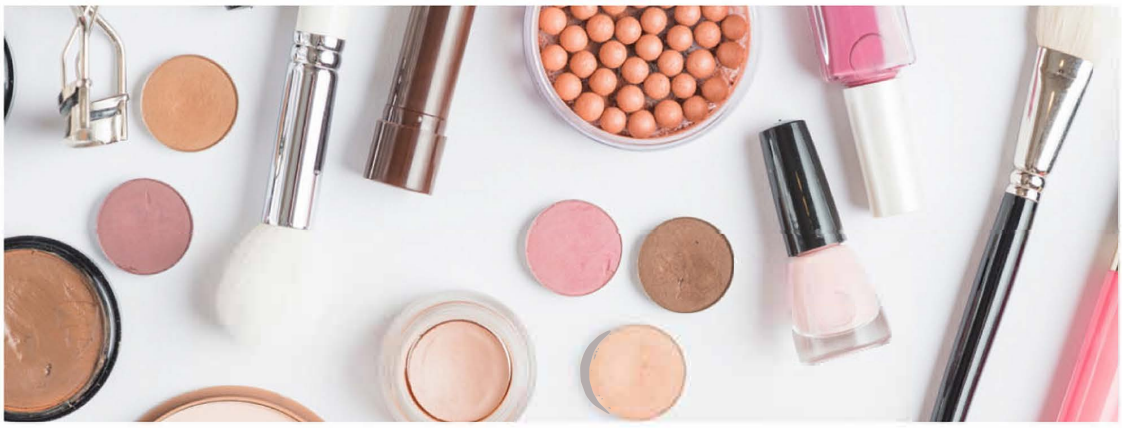
Disclaimer

ChemLinked provides translation services to assist clients in understanding the content of regulatory documents. We strive for the accuracy and reliability of our translations, but we do not warrant the accuracy, completeness, or suitability of the translated content.

Clients are responsible for the risks inherent in utilizing our translation services and should consult with professional legal advisors as required. ChemLinked disclaims any liability for any losses or damages arising from the use of our translation services.

Nondisclosure:
You may not disclose this document to anyone else without the written permission of ChemLinked.

For further clarification and questions, you can read our Privacy Policies or contact us at contact@chemlinked.com



You can now catch up with the latest updates of China CSAR on ChemLinked:

Stay tuned for more translations:
We will provide English translations of CSAR and supporting rules.

Watch our webinars:
Interpretation of China's Cosmetic Supervision and Administration Regulation

Check out the breaking news:
China Finalises the Long-Awaited Cosmetic Supervision and Administration Regulation

Visit the featured page for more information:
Featured — China Cosmetic Regulatory Reform Tracking

— CSAR —

Attachment 6

Bacterial Reverse Mutation Test

1 Scope

This method specifies the basic principles, requirements and methods for bacterial reverse mutation test.

This method applies to the testing of gene mutations in cosmetic ingredients and their products.

2 Definitions

2.1 Gene Mutation

A change in the sequence of base pairs in DNA under the action of a chemical mutagen.

2.2 Reverse Mutation

The reversion of bacteria from auxotroph to prototroph under the action of a chemical mutagen.

2.3 Bacterial Reverse Mutation Assay

A test method using histidine- or tryptophan-requiring mutant strains to determine the reversion of amino acid auxotrophs to prototrophs induced by chemical substances that cause base substitution or frameshift mutations in bacteria.

3 Principles

Histidine-dependent auxotrophic *Salmonella typhimurium* cannot synthesize histidine; therefore, on a histidine-free medium, only a small number of spontaneously reverting bacteria can grow. Tryptophan-dependent auxotrophic *Escherichia coli* cannot synthesize tryptophan; therefore, on a tryptophan-free medium, only a small number of spontaneously reverting bacteria can grow. In the presence of a mutagen, auxotrophic bacteria revert to prototrophs, allowing colony formation, which is used to determine whether the test substance is mutagenic.

Certain mutagens require metabolic activation to induce reverse mutations; therefore, an

S_9 mixture prepared from rat liver induced by an enzyme inducer is added.

4 Apparatus and Equipment

Incubator, constant temperature water bath, shaking water bath, pressure steam sterilizer, dry heat oven, ultra-low temperature freezer (-80 ℃) or liquid nitrogen biological container, laboratory refrigerator, balance, mixing oscillator, homogenizer, colony counter, low-temperature high-speed centrifuge, glassware, biosafety cabinet, *etc.*

5 Culture Media and Reagents

5.1 0.5 mmol/L Histidine - 0.5 mmol/L Tryptophan - 0.5 mmol/L Biotin Solution

Composition:	L-Histidine (MW155)	78 mg
	D-Biotin (MW244)	122 mg
	L-Tryptophan (MW204)	102 mg
	Add distilled / deionized water to	1,000 mL

Preparation: Heat all the above components to dissolve the biotin, and then autoclave at 115 ℃ for 20 min. Store in a refrigerator at 4 ℃. If *Escherichia coli* is not used in the test, do not add tryptophan.

5.2 Top Agar Medium

Composition:	Agar powder	1.2 g
	Sodium chloride	1.0 g
	Add distilled / deionized water to	200 mL

Preparation: Mix all the above components and autoclave at 121 ℃ for 30 min. During the test, add 20 mL of 0.5 mmol/L histidine-0.5 mmol/L tryptophan-0.5 mmol/L biotin solution. If *Escherichia coli* is not used in the test, do not add tryptophan.

5.3 Vogel-Bonner (V-B) Medium E

Composition:	Citric acid ($C_6H_8O_7 \cdot H_2O$)	100 g

Make Sure Your Products Entering Global Market with Compliance

What we can do for you?



+ Responsible person (RP) service in China
+ Cosmetic formula and label compliance review
+ Product registration and notification
+ New cosmetic ingredient (NCI) registration and notificaion
+ Ingredient safety information submission
+ Cosmetic efficacy evaluation



+ Cosmetic formula and label compliance review
+ EDI Notification
+ Cosmetic QC test service
+ Functional cosmetic registration
+ Ingredients registration by ICID



+ Marketing license application
+ Cosmetics notification
+ Ingredients analysis and full ingredient list translation
+ Cosmetic test service
+ Label & advertisement review



+ Responsible Person (RP) service in the EU & UK
+ Cosmetic Product Notification Portal (CPNP)
+ Cosmetic Product Safety Report (CPSR)
+ Preparation of Product Information File (PIF)



+ Cosmetic formula and label compliance review
+ FDA Voluntary Cosmetic Registration Program (VCRP)
+ California Safe Cosmetics Program (CSCP)
+ Toxicological Risk Assessment (TRA)
+ INCI application

What makes us unique?

- ☑ Connect with officials and associations
- ☑ Flexible consulting service packages
- ☑ Team of qualified toxicology and regulatory experts

CONTACT US | ☎ +353 1 8899 951 / +86 (0)571 8609 4444 ✉ cosmetic@chemlinked.com

附件 6

细菌回复突变试验方法

Bacterial Reverse Mutation Test

1 范围

本方法规定了细菌回复突变试验的基本原则、要求和方法。

本方法适用于化妆品原料及其产品的基因突变检测。

2 定义

2.1 基因突变 gene mutation

在化学致突变物作用下 DNA 中碱基对的排列顺序发生变化。

2.2 回复突变 reverse mutation

细菌在化学致突变物作用下由营养缺陷型回变到原养型（prototroph）。

2.3 细菌回复突变试验 bacterial reverse mutation assay

利用一组组氨酸或者色氨酸缺陷型试验菌株测定引起细菌碱基置换或移码突变的化学物质所诱发的氨基酸缺陷型回复突变为原养型的试验方法。

3 原理

组氨酸营养缺陷型鼠伤寒沙门氏菌不能合成组氨酸，故在缺乏组氨酸的培养基上，仅少数自发回复突变的细菌生长；色氨酸营养缺陷型大肠杆菌不能合成色氨酸，故在缺乏色氨酸的培养基上，仅少数自发回复突变的细菌生长。假如有致突变物存在，则营养缺陷型的细菌回复突变成原养型，因而能生长形成菌落，据此判断受试物是否为致突变物。

某些致突变物需要代谢活化后才能引起回复突变，故需加入经诱导剂诱导的大鼠肝制备的 S_9 混合液。

4 仪器和设备

培养箱、恒温水浴、振荡水浴摇床、压力蒸汽消毒器、干热烤箱、超低温冰箱（-80 ℃）或液氮生物容器、普通冰箱、天平、混匀振荡器、匀浆器、菌落计数器、低温高速离

心机、玻璃器皿、生物安全柜等。

5 培养基和试剂

5.1 0.5 mmol/L 组氨酸 - 0.5 mmol/L 色氨酸 - 0.5 mmol/L 生物素溶液

成分：	L-组氨酸（MW155）	78 mg
	D-生物素（MW244）	122 mg
	L-色氨酸（MW204）	102 mg
	加蒸馏水/去离子水至	1000 mL

配制：将上述成分加热，以溶解生物素，然后 115 ℃高压灭菌 20 min。储存于 4 ℃冰箱。若试验中不使用大肠杆菌，则不添加色氨酸。

5.2 顶层琼脂培养基

成分：	琼脂粉	1.2 g
	氯化钠	1.0 g
	加蒸馏水/去离子水至	200 mL

配制：上述成分混合后，121 ℃高压灭菌 30 min。试验时，加入 0.5 mmol/L 组氨酸 - 0.5 mmol/L 色氨酸 - 0.5 mmol/L 生物素溶液 20 mL。若试验中不使用大肠杆菌，则不添加色氨酸。

5.3 Vogel-Bonner（V-B）培养基 E

成分：	枸橼酸（$C_6H_8O_7 \cdot H_2O$）	100 g
	磷酸氢二钾（K_2HPO_4）	500 g
	磷酸氢铵钠（$NaNH_4HPO_4 \cdot 4H_2O$）	175 g
	硫酸镁（$MgSO_4 \cdot 7H_2O$）	10 g
	加蒸馏水/去离子水至	1000 mL

配制：先将前三种成分加热溶解后，再将溶解的硫酸镁缓缓倒入容量瓶中，加蒸馏水/



CHEMLINKED
by REACH24H Consulting Group

SUBSCRIBE TO CHEMLINKED NOW

REMOVE COSMETIC REGULATORY BARRIERS EXPEDITE GLOBAL MARKET ACCESS

- In-time regulatory information and market access requirements
- Supported by local and experienced technically adept technical team
- Strategic partnerships with competent authorities and industrial associations worldwide
- Customized consulting service for complete compliance solutions.

OUR SERVICES

Information & Knowledge

News, Reports, Expert Analysis
Pedia-articles

Databases

Regulatory Database, English Translations
Inventories/Lists

Solutions

Regulatory Compliance Service, On-demand Translation, Advertisement Service,Tailor-made Service, E-commerce Solution

Training

Webinars, Online Course
Offline Events

CHEMLINKED.COM

Paramount Court, Corrig Road, Sandyford, Dublin 18, Ireland
Tel: +353 1 8899 951 / +86 (0)571 8609 4444 / E-mail: cosmetic@chemlinked.com
Visit our website for more information about global cosmetic news & regulation updates