SLOVENSKI STANDARD
SIST ISO 16240:2007

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Kakovost vode - Določanje genotoksičnosti vode in odpadne vode - Preskus s Salmonella (preskus po Amesu)

Water quality -- Determination of the genotoxicity of water and waste water -- Salmonella/microsome test (Ames test)

Qualité de l'eau -- Détermination de la génotoxicité des eaux et des eaux résiduaires -- Essai de Salmonella/microsome (essai d'Ames)

Ta slovenski standard je istoveten z: ISO 16240:2005

ICS:

13.060.30	Odpadna voda	Sewage water
13.060.70	Preiskava bioloških lastnosti vode	Examination of biological properties of water

SIST ISO 16240:2007 **en**

INTERNATIONAL STANDARD

ISO 16240

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Water quality — Determination of the genotoxicity of water and waste water — *Salmonella*/microsome test (Ames test)

Qualité de l'eau — Détermination de la génotoxicité des eaux et des eaux résiduaires — Essai de Salmonella*/microsome (essai d'Ames)*

Reference number
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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 16240 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 5, *Biological methods*.

Introduction

It should be decided on a case-by-case basis whether, and to what extent, additional instructions may be necessary for the application of this International Standard.

Water quality — Determination of the genotoxicity of water and waste water — *Salmonella*/microsome test (Ames test)

WARNING — Persons using this International Standard should be familiar with normal laboratory practice. This International Standard does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices and to ensure compliance with any national regulatory conditions.

IMPORTANT — It is absolutely essential that tests conducted according to this International Standard be carried out by suitably trained staff.

1 Scope

This International Standard specifies a method for the determination of the genotoxic potential of water and wastewater using the bacterial strains *Salmonella typhimurium* TA 100 and TA 98. This method includes sterile filtration of water and wastewater prior to the test.

This International Standard is applicable only to the detection of genotoxic substances which are in the filtered aqueous phase. It is not applicable to the detection of genotoxic substances adsorbed by the retained particles.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 5667-1, *Water quality — Sampling — Part 1: Guidance on the design of sampling programmes*

ISO 5667-2, *Water quality — Sampling — Part 2: Guidance on sampling techniques*

ISO 5667-3, *Water quality — Sampling — Part 3: Guidance on the preservation and handling of water samples*

ISO 5667-14, *Water quality — Sampling — Part 14: Guidance on quality assurance of environmental water sampling and handling*

ISO 5667-16, *Water quality — Sampling — Part 16: Guidance on biotesting of samples*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
number of revertants
number of mutants
number of visible mutant colonies per plate at the termination of the test

3.2
dilution level
D
denominator of the dilution coefficient (using the numerator 1) of a mixture of water or wastewater with **dilution water** (3.16) as integral number

NOTE For undiluted water or wastewater, the dilution coefficient is by definition 1:1. The corresponding and smallest possible D value is 1.

3.3
dose-response relationship
reduction of the number of visible mutant colonies per plate with increasing D level

3.4
D_{min} **value**
smallest value of D at which, under the conditions of this International Standard, no positive increase in the number of visible mutant colonies per plate is detected

NOTE In the case of more than one D_{min} value (a maximum of four are possible), the highest D value is decisive.

3.5
stock culture
frozen culture for the preservation of the characteristics (e.g. genotype) of *Salmonella typhimurium* TA 100 and TA 98

3.6
inoculum
part of a thawed stock culture used to inoculate culture medium

3.7
culture medium
aqueous solution of nutrients which are required for the cultivation of the bacteria

3.8
overnight culture
mixture of inoculum and culture medium, incubated for about 18 h at 37 °C ± 1 °C and gentle agitation (e.g. shaken at 100 r/min to 150 r/min)

3.9
plate
solidified mixture of water, agar and other possible constituents (e.g. inorganic salts) in Petri dishes

3.10
softagar
mixture of agar, sodium chloride, histidine, biotin and water

NOTE Minimal softagar contains only traces of histidine and is used for the determination of mutants. Maximal softagar contains histidine in excess and is used for the determination of titres.

3.11
S9 fraction
9 000 g supernatant of a tissue homogenate in 0,15 mol/l KCl, obtained from livers of male rats (200 g to 300 g) pretreated with an appropriate substance or substance combination for enzyme induction

3.12
cofactor solution
aqueous solution of chemicals needed for the activity of the enzymes in the S9 fraction

NOTE Examples of chemicals needed are NADP, glucose-6-phosphate and inorganic salts.

3.13
S9 mix
mixture of S9 fraction and cofactor solution

3.14
titre determination
method for the determination of the number of bacteria (colony-forming units) in an overnight culture and for the determination of possible bacteriotoxic effects of the test sample

3.15
test sample
sample to be used as test item after all preparative steps (e.g. sterile filtration) have been carried out

3.16
dilution water
sterile water of a conductivity of $\leqslant$ 5 µS/cm used for the stepwise dilution of the test sample or used as negative control

3.17
negative control
dilution water (3.16) without test sample

3.18
positive control
known mutagen used to verify the sensitivity of the method or the activity of the S9 mix

NOTE The positive controls are dissolved in DMSO prior to use.

3.19
test mixture
mixture of test sample [pure or diluted with **dilution water** (3.16)], negative or positive control, bacterial suspension, softagar and S9 mix or buffer

3.20
induction rate
I
difference between the mean value of mutant colonies counted on the plates treated with a dose of the test sample or with a positive control and the mean value of the corresponding plates treated with the negative control using the same strain under identical activation conditions

3.21
background growth
bacterial lawn formed by microcolonies of non-mutated bacteria on a plate with minimal softagar due to the traces of histidine contained in this softagar

4 Interferences

A strong bacteriotoxic effect of the test sample can lead to a reduction of viable bacteria and to a reduction of mutant colonies compared to the corresponding negative control counts.

In an extreme case of bacteriotoxicity, the number of surviving bacteria may be reduced to such an extent (to several hundred) that the traces of histidine in the minimal softagar are sufficient to allow these bacteria to grow up to visible colonies mimicking the growth of mutant colonies. This may lead to false positive results.