SLOVENSKI STANDARD
SIST ISO 13829:2010

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Kakovost vode - Določevanje genotoksičnosti vode in odpadne vode z umu-preskusom

Water quality - Determination of the genotoxicity of water and waste water using the umu -test

Qualité de l'eau - Détermination de la génotoxicité des eaux et des eaux résiduaires à l'aide de l'essai umu

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INTERNATIONAL STANDARD

ISO 13829

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Water quality — Determination of the genotoxicity of water and waste water using the umu-test

Qualité de l'eau — Détermination de la génotoxicité des eaux et des eaux résiduaires à l'aide de l'essai umu

Reference number
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Contents

Page

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 3.

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 International Standard may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

International Standard ISO 13829 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 5, *Biological methods*.

Annexes A to G of this International Standard are for information only.

Introduction

The genetically engineered bacterium *Salmonella typhimurium* TA1535/pSK1002 serves as a test organism.

The bacteria are exposed under controlled conditions to different concentrations of the samples to be tested. The test is based on the capability of genotoxic agents to induce the umuC-gene in the *Salmonella* strain in response to genotoxic lesions in the DNA.

Due to its capability to respond to different types of genotoxic lesions, only one single strain is necessary to detect different kinds of genotoxic substances.

The induction of the umuC-gene is thus a measure for the genotoxic potential of the sample. Since the umuC-gene is fused with the lacZ-gene for β-galactosidase, the induction of the umuC-gene can be easily assessed by determination of the β-galactosidase activity.

Water quality — Determination of the genotoxicity of water and waste water using the umu-test

WARNING — This test involves the use of genetically modified organisms. National or international licensing may restrict the use of these organisms.

Test conducted according to this International Standard should be carried out by qualified experts or by a qualified testing laboratory.

When applying this International Standard it is necessary in each case, depending on the range to be tested, to determine if and to which extent additional criteria should be established.

1 Scope

This International Standard specifies a procedure which can be used to determine the genotoxicity[1)] of water and waste water using the umu-test.

This assay is based on the detection of genotoxicity of a test sample which increases the expression of the SOS-repair system[2)] associated with the umuC-gene[3)].

2 Normative reference

The following normative document contains provisions which, through reference in this text, constitute provisions of this International Standard. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent edition of the normative document indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

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

3 Terms and definitions

For the purposes of this International Standard, the following terms and definitions apply. Other related terms and definitions have been included in annex A for information.

3.1
stock culture
culture of a bacterial strain to preserve the original test strain and to prepare the inoculation material for the overnight culture or the pre-culture

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1) Toxicity which specifically affects the genome (genetic material).

2) SOS repair occurs when cells are overwhelmed by genotoxins allowing the cell to survive at the cost of mutagenesis.

3) umuC-gene is the acronym for UV mutagenesis gene C. The induction of the umuC-gene is part of the specific response of the bacterial cell to DNA-damage.

3.2
overnight culture
culture of test bacteria for the preparation of the pre-culture

3.3
pre-culture
culture for adaptation of the overnight culture to the test conditions and to prepare the inoculum for the assay

3.4
inoculum
inoculation material
aliquot of a bacterial suspension used for inoculation in the assay

3.5
concentration effect relationship
induction of the umuC-gene depending on the concentration of genotoxic agents in the test sample

3.6
culture medium
aqueous solution of nutrients required for bacterial growth

3.7
test sample
the sample to be tested, after finishing all preparations

EXAMPLES Preparations may include centrifugation, filtration, homogenization, pH adjustment and measurement of conductivity.

3.8
dilution series
mixture of the test sample and dilution water in varying proportions

3.9
test mixture
mixture of culture medium, inoculum and dilution series

3.10
negative control
culture medium

3.10.1
blank
culture medium without bacteria

3.10.2
negative control for test samples
mixture of culture medium, inoculum and distilled water

3.10.3
solvent control
mixture of culture medium, inoculum and dimethyl sulfoxide

3.11
positive control
mixture of culture medium, inoculum and a dissolved genotoxic substance

EXAMPLES Typical genotoxic substances are 4-nitroquinoline-*N*-oxide or 2-aminoanthracene in the case of metabolic activation.

3.12
S9 fraction
⟨metabolic activation system⟩ 9 000 *g* centrifugation supernatant prepared from the livers of male rats pretreated with enzyme-inducing agents

NOTE Bacteria are exposed to the test sample both with and without an appropriate metabolic activation system.

4 Principle

The test organisms are exposed to the test sample with and without metabolic activation system using microplates. After 4 h of incubation, the genotoxin-dependent induction of the umuC-gene is compared to the spontaneous activation of the untreated, control culture.

5 Test organism and reagents

5.1 Test organism and stock culture

5.1.1 Test organism

Salmonella typhimurium is a gram-negative, facultative, anaerobic bacterium from the *Enterobacteriaceae* family. *Salmonella typhimurium* TA1535 is the original strain. The test organism carries the plasmid pSK1002 with the umuC-lacZ gene and a gene for ampicillin resistance. The designation of this *Salmonella* strain is "TA1535/pSK1002" (see annex B). This bacterial strain can be easily selected due to its ampicillin resistance.

5.1.2 Stock culture preparation and preservation

Preserve *Salmonella typhimurium* TA1535/pSK1002 in 150 µl culture medium with 10 % dimethyl sulfoxide (DMSO) or 20 % glycerol in 2 ml ampoules at a temperature not above – 80 °C. For the preparation of an overnight culture only one ampoule is used.

5.2 Reagents

Chemicals shall be of analytical grade. Prepare all solutions with purified deionized water or water of equivalent purity.

5.2.1 Hydrochloric acid, *c*(HCl) = 1mol/l.

5.2.2 Sodium hydroxide solution, *c*(NaOH) = 1 mol/l.

5.2.3 Dimethyl sulfoxide (DMSO), $C_2H_6SO_4$.

WARNING — DMSO forms mutagenic products over a period of time.

5.2.4 TGA-culture medium, consisting of tryptone, glucose and ampicillin, prepared as follows.

Dissolve 10 g of tryptone, 5 g of sodium chloride (NaCl) and 11,9 g of 4-(2-hydroxyethyl)-*l*-piperazineethanesulphonic acid (HEPES) in water, adjust the pH-value to 7,0 ± 0,2, dilute to 980 ml and autoclave for 20 min at 121 °C. Dissolve 2 g of *D*(+)-glucose (anhydrous) in 20 ml distilled water and autoclave separately. After autoclaving, mix the two solutions in equal proportions and add 50 mg of ampicillin to 1 000 ml of cooled TGA medium under sterile conditions. The solution can be stored in portions at – 20 °C for up to 4 weeks.

5.2.5 Concentrated 10× TGA-culture medium, consisting of a tenfold concentrated TGA (5.2.4) solution, which can be stored for 14 days at 4 °C.