

SLOVENSKI STANDARD
oSIST prEN ISO 10253:2016
01-februar-2016

Kakovost vode - Preskus zaviranja rasti morskih alg s *Skeletonema* sp. in *Phaeodactylum tricornutum* (ISO/DIS 10253:2015)

Water quality - Marine algal growth inhibition test with *Skeletonema* sp. and *Phaeodactylum tricornutum* (ISO/DIS 10253:2015)

Wasserbeschaffenheit - Wachstumshemmtest mit marinen Algen *Skeletonema costatum* und *Phaeodactylum tricornutum* (ISO/DIS 10253:2015)

Qualité de l'eau - Essai d'inhibition de la croissance des algues marines avec *Skeletonema* sp. et *Phaeodactylum tricornutum* (ISO/DIS 10253:2015)

Ta slovenski standard je istoveten z: prEN ISO 10253

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ICS:

13.060.70	Preiskava bioloških lastnosti vode	Examination of biological properties of water
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DRAFT INTERNATIONAL STANDARD

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Water quality — Marine algal growth inhibition test with *Skeletonema* sp. and *Phaeodactylum tricornutum*

*Qualité de l'eau — Essai d'inhibition de la croissance des algues marines avec *Skeletonema* sp. et *Phaeodactylum tricornutum**

ICS: 13.060.70

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ISO/CEN PARALLEL PROCESSING

This draft has been developed within the International Organization for Standardization (ISO), and processed under the **ISO lead** mode of collaboration as defined in the Vienna Agreement.

This draft is hereby submitted to the ISO member bodies and to the CEN member bodies for a parallel five month enquiry.

Should this draft be accepted, a final draft, established on the basis of comments received, will be submitted to a parallel two-month approval vote in ISO and formal vote in CEN.

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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.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information

The committee responsible for this document is ISO/TC 147, *Water quality*, Subcommittee 5, *Biological methods*.

This third edition cancels and replaces the second edition (ISO 10253:2006), which has been technically revised as follows:

Inclusion of new Annex B (informative) "Test procedure starting from stored algal inocula, and with direct measurement of algal growth in spectrophotometric cells".

Water quality — Marine algal growth inhibition test with *Skeletonema* sp. and *Phaeodactylum tricornutum*

WARNING — Persons using this document should be familiar with normal laboratory practice. This document 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 document be carried out by suitably trained staff.

1 Scope

This International Standard specifies a method for the determination of the inhibition of growth of the unicellular marine algae *Skeletonema* sp. and *Phaeodactylum tricornutum* by substances and mixtures contained in sea water or by environmental water samples (effluents, elutriates, etc).

The method can be used for testing substances that are readily soluble in water and are not significantly degraded or eliminated in any other way from the test medium.

NOTE With modifications, as described in ISO 14442 and ISO 5667-16, the inhibitory effects of poorly soluble organic and inorganic materials, volatile compounds, metal compounds, effluents, marine water samples and elutriates of sediments can be tested.

2 Normative references

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

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

ISO 14442, *Water quality — Guidelines for algal growth inhibition tests with poorly soluble materials, volatile compounds, metals and waste water*

3 Terms and definitions

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

3.1

cell density

number of cells per unit volume of medium (x cells/ml)

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3.2

specific growth rate μ

proportional rate of increase in cell density per unit of time:

$$\mu = \frac{1}{x} \times \frac{dx}{dt} \text{ (1/day)}$$

where

x is the cell density, expressed in cells per millilitre;

t is the time, expressed in days.

Note 1 to entry: Specific growth rate is expressed in inverse days (day^{-1}).

3.3

growth medium

mixture of sea water and nutrients which is used for pre-cultures and controls

3.4

test medium

mixture of sea water, nutrients (growth medium (3.3)) and test material in which algal cells are incubated

3.5

test batch

mixture of sea water, nutrients and test material (test medium (3.4)) inoculated with algae

3.6

control

mixture of sea water, nutrients (growth medium (3.3)) without test material, inoculated with algae

3.7

effective concentration $EC(r)_x$

concentration of test substance which results in an x % reduction in specific growth rate relative to the controls

4 Principle

Mono-specific algal strains are cultured for several generations in a defined medium containing a range of concentrations of the test substance, prepared by mixing appropriate quantities of nutrient concentrate, sea water, stock solutions of the test substance, and an inoculum of exponentially growing algal cells. The test solutions are incubated for a period of $72 \text{ h} \pm 2 \text{ h}$, during which the cell density in each is measured at intervals of at least every $24 \text{ h} \pm 2 \text{ h}$. Inhibition is measured as a reduction in specific growth rate, relative to control cultures grown under identical conditions.

5 Materials

5.1 Test organisms

Use either of the following marine algae:

- a) *Skeletonema* sp.¹.(CCAP 1077/1C, NIVA BAC 1); or
- b) *Phaeodactylum tricornutum* Bohlin (CCAP 1052/1A, SAG 1090-1a, NIVA BAC 2).

These algae are important and widely distributed phytoplankton species (phylum *Bacillariophyta*) in estuarine and coastal areas.

The recommended algae are available in unialgal, non-axenic cultures from the following sources.

NIVA
Norwegian Institute for Water Research
Gaustadaléen 21
N 0349 Oslo
Norway

CCAP
Dunstaffnage Marine Laboratory
P O Box 3 Oban
Argyll PA37 1QA
United Kingdom

SAG
Collection of Algal Cultures
University of Göttingen
Albrecht-von-Haller Institute for Plant Science
Untere Karspüle 2
37073 Göttingen
Germany

Stock cultures may be maintained in the medium described in 7.1. Regular subculturing is necessary. Weekly intervals may be necessary for *Skeletonema* sp., every two or three weeks may be sufficient for *Phaeodactylum tricornutum*. The stock cultures may also be maintained for extended periods on richer algal media such as those recommended by the culture collection. It is recommended to keep the stock culture in the medium described in 7.1 and in an exponential growth phase immediately before preparing the pre-culture for testing as described in 7.2.

NOTE Concentrated cultures of the diatom *Phaeodactylum tricornutum* can also be stored for several months without losing their viability. Stock cultures for the toxicity tests can easily be prepared from the stored concentrated cultures²⁾.

¹ The previous editions of this International Standard suggested the use of two strains of *Skeletonema costatum*. Following a taxonomic review of the *Skeletonema* genus, several strains originally identified as *S. costatum* may in fact be other species. In light of this and to enable continuity in the use of previously accepted strains, the present revision of ISO 10253 has changed the reference from *Skeletonema costatum* to *Skeletonema* sp. to avoid non-compliance for labs that may be using different strains.

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5.2 Water

All water used in the preparation of the synthetic sea water, growth medium and test substance solutions shall be deionized or of equivalent purity. Take special care to avoid contamination of the water by inorganic or organic substances during preparation and storage. Equipment made of copper shall not be used.

5.3 Sea water

For culturing and testing *Phaeodactylum tricornutum*, the growth medium (7.1) is made up by adding nutrients to either natural [salinity = (30 ± 5) g/kg] or synthetic sea water (approximate salinity = 33 g/kg). For *Skeletonema* sp., the use of natural sea water may be necessary for the long-term maintenance of cultures and may also be necessary for the test medium, because a synthetic sea water medium may not always support sufficient growth to meet the test quality criteria. If natural sea water is used, care shall be taken to ensure that it is not polluted.

Prepare synthetic sea water with the composition given in Table 1 (approximate salinity = 33 g/kg). All the chemicals used shall be of analytical grade.

Table 1 — Synthetic sea water

Salt	Concentration of salt in synthetic sea water
	g/l
NaCl	22
MgCl ₂ ·6H ₂ O	9,7
Na ₂ SO ₄ (anhydrous)	3,7
CaCl ₂ (anhydrous)	1,0
KCl	0,65
NaHCO ₃	0,20
H ₃ BO ₃	0,023

Filter the sea water (synthetic as well as natural one) through a 0,45 µm membrane filter in order to remove particulate material and algae.

5.4 Nutrients

Prepare three nutrient stock solutions in water, with the compositions given in Table 2.

2) Concentrated *Phaeodactylum tricornutum* cultures can be supplied by MicroBioTests Inc. Mariakerke, Belgium. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product. Equivalent products may be used if they can be shown to lead to the same results.