



SLOVENSKI STANDARD
SIST EN ISO 10253:2006
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BUXca Yý U.
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Kakovost vode – Preskus zaviranja rasti morskih alg s *Skeletonema costatum* in *Phaeodactylum tricornutum*

Water quality - Marine algal growth inhibition test with *Skeletonema costatum* and *Phaeodactylum tricornutum* (ISO 10253:2006)

Wasserbeschaffenheit - Wachstumshemmtest mit marinen Algen *Skeletonema costatum* und *Phaeodactylum tricornutum* (ISO 10253:2006)

Qualité de l'eau - Essai d'inhibition de la croissance des algues marines avec *Skeletonema costatum* et *Phaeodactylum tricornutum* (ISO 10253:2006)

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English Version

Water quality - Marine algal growth inhibition test with
Skeletonema costatum and Phaeodactylum tricornutum (ISO
10253:2006)

Qualité de l'eau - Essai d'inhibition de la croissance des
algues marines avec *Skeletonema costatum* et
Phaeodactylum tricornutum (ISO 10253:2006)

Wasserbeschaffenheit - Wachstumshemmtest mit marinen
Algen (*Skeletonema costatum* und
Phaeodactylum tricornutum) (ISO 10253:2006)

This European Standard was approved by CEN on 13 April 2006.

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the Central Secretariat or to any CEN member.

This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the Central Secretariat has the same status as the official versions.

CEN members are the national standards bodies of Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland and United Kingdom.

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

Management Centre: rue de Stassart, 36 B-1050 Brussels

Foreword

This document (EN ISO 10253:2006) has been prepared by Technical Committee ISO/TC 147 "Water quality" in collaboration with Technical Committee CEN/TC 230 "Water analysis", the secretariat of which is held by DIN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by October 2006, and conflicting national standards shall be withdrawn at the latest by October 2006.

This document supersedes EN ISO 10253:1998.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland and United Kingdom.

Endorsement notice

The text of ISO 10253:2006 has been approved by CEN as EN ISO 10253:2006 without any modifications.

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

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

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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 10253 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 5, *Biological methods*.

This second edition cancels and replaces the first edition (ISO 10253:1995), which has been technically revised.

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Water quality — Marine algal growth inhibition test with *Skeletonema costatum* and *Phaeodactylum tricornerutum*

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 inhibition of growth of the unicellular marine algae *Skeletonema costatum* and *Phaeodactylum tricornerutum* by substances and mixtures contained in sea water.

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 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 5667-16, *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)

**3.2
specific growth rate**

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

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

**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

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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 costatum* (Greville) Cleve (CCAP 1077/1C, NIVA BAC 1); or
- b) *Phaeodactylum tricorutum* 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 strains recommended are available in unialgal, non-axenic cultures from the following sources.

NIVA	Norwegian Institute for Water Research P.O Box 173 Kjelsås N-0411 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*; every two or three weeks may be sufficient for *Phaeodactylum*. 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.

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NOTE Like many freshwater algae, the diatom *Phaeodactylum tricornutum* can also be stored for several months in alginate beads, without losing its viability^[1]. The algae can be liberated from the algal beads when needed to perform the toxicity tests¹⁾.

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5.2 Water

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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*, 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*, 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.

1) The algae beads supplied by MICROBIOTESTS Inc., Venecoweg 19, 9810 Nazareth, Belgium, Tel (32) 9 380 8545, Fax (32) 9 380 8546, Email microbiotests@skynet.be, are an example of a suitable commercially available product. 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.