
**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**

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Reference number
ISO 10253:2016(E)

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Contents

Page

Foreword	iv
1 Scope	1
2 Normative references	1
3 Terms and definitions	1
4 Principle	2
5 Materials	3
5.1 Test organisms	3
5.2 Reagents	4
6 Apparatus	5
7 Procedure	6
7.1 Preparation of growth medium	6
7.2 Preparation of pre-culture and inoculum	6
7.3 Choice of test concentrations	6
7.4 Preparation of test substance stock solutions	6
7.5 Preparation of test and control batches	7
7.6 Incubation	7
7.7 Measurements	7
8 Validity criteria	8
9 Interpretation of data	8
9.1 Plotting growth curves	8
9.2 Calculation of percentage inhibition	8
9.3 Determination of $EC(r)_x$	9
10 Expression of results	9
11 Interpretation of results	9
12 Test report	9
Annex A (informative) Preparation of dilution series of mixtures in sea water (effluents or elutriates)	11
Annex B (informative) Test procedure starting from stored algal inocula, and with direct measurement of algal growth in spectrophotometric cells	12
Annex C (informative) Performance data	18
Bibliography	19

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 World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see the following URL: www.iso.org/iso/foreword.html.

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

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

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 document 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 are referred to in the text in such a way that some or all of their content constitutes requirements 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.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- IEC Electropedia: available at <http://www.electropedia.org/>
- ISO Online browsing platform: available at <http://www.iso.org/obp>

3.1

cell density

number of cells per unit volume of medium

Note 1 to entry: The cell density is expressed as x cells/ml.

3.2 specific growth rate

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

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

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 ± 2) h, during which the cell density in each is measured at intervals of at least every (24 ± 2) 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

1) The previous editions of this document 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 this document has changed the reference from *Skeletonema costatum* to *Skeletonema* sp. to avoid non-compliance for labs that may be using different strains.