

BS EN ISO 8692:2012



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Water quality — Fresh water algal growth inhibition test with unicellular green algae (ISO 8692:2012)

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National foreword

This British Standard is the UK implementation of EN ISO 8692:2012. It supersedes BS EN ISO 8692:2004, which is withdrawn.

The UK participation in its preparation was entrusted to Technical Committee EH/3/5, Biological Methods.

A list of organizations represented on this committee can be obtained on request to its secretary.

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English Version

Water quality - Fresh water algal growth inhibition test with unicellular green algae (ISO 8692:2012)

Qualité de l'eau - Essai d'inhibition de la croissance des algues d'eau douce avec des algues vertes unicellulaires (ISO 8692:2012)

Wasserbeschaffenheit - Süßwasseralgen-Wachstumshemmtest mit einzelligen Grünalgen (ISO 8692:2012)

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EUROPÄISCHES KOMITEE FÜR NORMUNG

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Foreword

This document (EN ISO 8692:2012) 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of an identical text or by endorsement, at the latest by August 2012, and conflicting national standards shall be withdrawn at the latest by August 2012.

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

This document supersedes EN ISO 8692:2004.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, 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, Turkey and the United Kingdom.

Endorsement notice

The text of ISO 8692:2012 has been approved by CEN as a EN ISO 8692:2012 without any modification.

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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 8692 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 5, *Biological methods*.

This third edition cancels and replaces the second edition (ISO 8692:2004), which has been technically revised.

Water quality — Fresh water algal growth inhibition test with unicellular green algae

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 in accordance with this International Standard be carried out by suitably qualified staff.

1 Scope

This International Standard specifies a method for the determination of the growth inhibition of unicellular green algae by substances and mixtures contained in water or by waste water. This method is applicable for substances that are easily soluble in water.

With modifications to this method, as specified in ISO 14442 and ISO 5667-16, the inhibitory effects of poorly soluble organic and inorganic materials, volatile compounds, heavy metals and waste water can be tested.

A rapid algal growth inhibition screening test for waste water is described in Annex A.

An alternative test procedure with algae from algal beads, with direct measurement of algal growth in spectrophotometric cells, is described in Annex B.

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/TR 11044, *Water quality — Scientific and technical aspects of batch algae growth inhibition tests*

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

ISO/TS 20281, *Water quality — Guidance on statistical interpretation of ecotoxicity data*

3 Terms and definitions

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

3.1 cell density

n

number of cells per volume of medium

NOTE Cell density is expressed in cells per millilitre.

3.2

effective concentration

concentration of the test sample (EC_x) at which an effect of x % is measured, if compared to the control

NOTE To unambiguously denote an EC value deriving from growth rate, it is proposed to use the symbol “ E_rC ”.

3.3

lowest ineffective dilution

LID

dilution level at which no inhibition, or only effects not exceeding the test-specific variability, are observed

NOTE Adapted from ISO 15088:2007^[13], 3.5.

3.4

specific growth rate

μ

proportional rate of increase in cell density per time:

$$\mu = \frac{1}{n} \frac{dn}{dt}$$

where

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

t is the time, expressed in days.

NOTE Specific growth rate is expressed in reciprocal days (day^{-1}).

[ISO/TR 11044:2008, 3.2]

4 Principle

Monospecies algal strains are cultured for several generations in a defined medium containing a range of concentrations of the test sample, prepared by mixing appropriate quantities of growth medium, test sample, and an inoculum of exponentially growing algal cells. The test batches are incubated for a period of (72 ± 2) h during which the cell density in each test solution is measured at least every 24 h.

Inhibition is measured as a reduction in specific growth rate relative to control cultures grown under identical conditions.

5 Reagents and media

5.1 Test organism, using either of the following planktonic fresh water algae species:

- a) *Desmodesmus subspicatus* (R. Chodat) E. Hegewald et A. Schmidt¹⁾ (86.81 SAG²⁾);
- b) *Pseudokirchneriella subcapitata* (Korshikov) Hindak³⁾ (ATCC® 22662TM,²⁾ CCAP 278/4²⁾ or 61.81 SAG²⁾).

NOTE 1 The two species do not show identical responses to toxic agents.

NOTE 2 Both algae species are planktonic green algae belonging to the order of Sphaeropleales (Chlorophyta, Chlorophyceae) and are usually unicellular in culture.

1) This species is formerly known as *Scenedesmus subspicatus* Chodat.

2) Trade names of strains are examples of suitable strains available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of these products.

3) This species is formerly known as *Selenastrum capricornutum* Prinz. The new name is currently cited by culture collections.

The strains recommended are available in unialgal, non-axenic cultures from the following collections:

- SAG — Sammlung von Algenkulturen Göttingen [Göttingen Algal Culture Collection], Germany
www.epsag.uni-goettingen.de (viewed 2012-01-30);
- ATCC — American Type Culture Collection, USA
www.atcc.org (viewed 2012-01-30);
- CCAP — Culture Collection of Algae and Protozoa, UK
www.ccap.ac.uk (viewed 2012-01-30);
- ALCP — Algorithèque du Laboratoire de Cryptogamie, France
www.mnhn.fr (viewed 2012-01-30).

Stock cultures can be maintained in the medium specified in 5.3. and 7.1. However, frequent subculturing is necessary (once a week) to prevent failure of growth. The stock culture can be maintained for extended periods on richer algal media such as those recommended by the culture collection.

Alternatively, algae can be stored for several months on agar plates or in alginate beads⁴⁾ without losing their viability^[1]. The algae can be easily recovered from the agar or liberated from the algal beads (see Annex C) when needed to perform the toxicity tests.

The appearance of the cells and the identity of the test organisms should be confirmed by microscopy.

5.2 Water, deionized or of equivalent purity (conductivity <10 µS/cm), for use in the preparation of the growth medium and test substance solutions.

Take special care to avoid contamination of the water by inorganic or organic substances during preparation and storage. Do not use equipment made of copper.

5.3 Nutrients

Prepare four nutrient stock solutions in water, according to the compositions given in Table 1.

These solutions are eventually diluted (see 7.1 and 7.4) to achieve the final nutrient concentrations in the test solutions. However, the macronutrients may instead be added directly to the water.

All chemicals used shall be of reagent-grade quality.

Sterilize the stock solutions by membrane filtration (mean pore diameter 0,2 µm) or by autoclaving [(120 ± 2) °C, 15 min]. Store the solutions in the dark at 4 °C.

Do not autoclave stock solution 4 in order to avoid evaporative loss of NaHCO₃, but sterilize it by membrane filtration.

4) The algal beads supplied by MicroBioTests Inc., Mariakerke-Gent, Belgium are an example of a suitable product available commercially. 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 the validity criteria specified in this document are fulfilled.