

**Water quality - Guidance on the use of in vivo
absorption techniques for the estimation of chlorophyll-
a concentration in marine and fresh water samples**

EESTI STANDARDI EESSÕNA

NATIONAL FOREWORD

See Eesti standard EVS-EN 16161:2012 sisaldab Euroopa standardi EN 16161:2012 ingliskeelset teksti.	This Estonian standard EVS-EN 16161:2012 consists of the English text of the European standard EN 16161:2012.
Standard on jõustunud sellekohase teate avaldamisega EVS Teatajas.	This standard has been endorsed with a notification published in the official bulletin of the Estonian Centre for Standardisation.
Euroopa standardimisorganisatsioonid on teinud Euroopa standardi rahvuslikele liikmetele kättesaadavaks 04.07.2012.	Date of Availability of the European standard is 04.07.2012.
Standard on kättesaadav Eesti Standardikeskusest.	The standard is available from the Estonian Centre for Standardisation.

Tagasisidet standardi sisu kohta on võimalik edastada, kasutades EVS-i veebilehel asuvat tagasiside vormi või saates e-kirja meiliaadressile standardiosakond@evs.ee.

ICS 13.060.70

Standardite reprodutseerimise ja levitamise õigus kuulub Eesti Standardikeskusele

Andmete paljundamine, taastekitamine, kopeerimine, salvestamine elektroonsesse süsteemi või edastamine ükskõik millises vormis või millisel teel ilma Eesti Standardikeskuse kirjaliku loata on keelatud.

Kui Teil on küsimusi standardite autorikaitse kohta, võtke palun ühendust Eesti Standardikeskusega:
Aru 10, 10317 Tallinn, Eesti; www.evs.ee; telefon 605 5050; e-post info@evs.ee

The right to reproduce and distribute standards belongs to the Estonian Centre for Standardisation

No part of this publication may be reproduced or utilized in any form or by any means, electronic or mechanical, including photocopying, without a written permission from the Estonian Centre for Standardisation.

If you have any questions about copyright, please contact Estonian Centre for Standardisation:
Aru 10, 10317 Tallinn, Estonia; www.evs.ee; phone 605 5050; e-mail info@evs.ee

English Version

Water quality - Guidance on the use of in vivo absorption techniques for the estimation of chlorophyll-a concentration in marine and fresh water samples

Qualité de l'eau - Lignes directrices sur l'utilisation des techniques d'absorption in vivo pour l'estimation de la concentration de chlorophylle-a dans les eaux douces et eaux marines

Wasserbeschaffenheit - Anleitung für die Anwendung der in-vivo-Absorption zur Abschätzung der Chlorophyll a-Konzentration in Meer- und Süßwasser

This European Standard was approved by CEN on 17 May 2012.

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 CEN-CENELEC Management Centre 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 CEN-CENELEC Management Centre has the same status as the official versions.

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



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

Management Centre: Avenue Marnix 17, B-1000 Brussels

Contents

Page

Foreword.....	3
Introduction	4
1 Scope	5
2 Normative references	5
3 Terms and definitions	5
4 Principle.....	6
5 Apparatus	6
6 Procedure	7
6.1 Calibration	7
6.2 Blank Measurement.....	7
6.3 Sample measurement.....	8
7 Calculation and Expression of Results	8
7.1 General.....	8
7.2 Datum.....	8
7.3 Mathematical routine.....	9
7.4 Other factors influencing the chlorophyll-a estimation.....	9
8 Quality Assurance	9
8.1 Repeatability.....	9
8.2 Uncertainty	9
9 Test report	10
Annex A (normative) Published <i>in vivo</i> specific chlorophyll a absorption spectrum	11
Annex B (informative) Determination of the appropriate chlorophyll-a specific spectral absorption coefficient for the IVP system	13
Annex C (informative) Factors influencing the chlorophyll-a estimation.....	16
C.1 Breakdown pigments	16
C.2 Package effect.....	17
C.3 Chlorophyll-b.....	18
C.4 Phycocyanin.....	18
Annex D (informative) Examples of paired sample method validation — Comparison of extraction and <i>in vivo</i> methods under operational conditions	20
D.1 General.....	20
D.2 The IRH Laboratory Data Set	20
D.3 The Adasa Sistemas Laboratory Data Set	21
Annex E (informative) Validation of the spectrometric technique by determining the chlorophyll-a specific absorption of a set of algal samples	22
E.1 Laboratory sample data for validation a chl_{sys} the appropriate system unit chlorophyll-a response peak height.....	22
E.2 <i>In vivo</i> photometric chlorophyll-a and the package effect.....	24
Bibliography	25

Foreword

This document (EN 16161:2012) has been prepared by 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 January 2013, and conflicting national standards shall be withdrawn at the latest by January 2013.

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.

According to the CEN/CENELEC Internal Regulations, the national standards organisations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Former Yugoslav Republic of Macedonia, 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.

Introduction

Surveys of chlorophyll and nutrient concentration are fundamental descriptors of primary productivity and eutrophic threat in coastal and inland waters.

Chlorophyll-a concentration can be determined by sampling and laboratory analysis using the techniques described in ISO 10260. Achieving consistent results with this technique requires careful attention during the various steps of the process commonly used, such as during sampling, transport, filtering, freezing, storage and extraction and subsequent pigment estimation.

The *in vivo* technique described here can be applied to surveys where a rapid non-destructive and repeatable measurement capability is required. It can be used either in the field or laboratory. No chemicals are required. Utilised in association with other methods of chlorophyll-a determination such as ISO 10260, HPLC pigment analysis and chlorophyll fluorescence measurements techniques, it can help identify sources of inconsistency or be used as an alternative technique in its own right. As chlorophyll-a estimates can be achieved in times as short as one minute, the technique can enhance surveying capability considerably.

This standard describes procedures to implement and verify performance.

1 Scope

This European Standard provides guidance in the use of *in vivo* absorption techniques to quantify chlorophyll-a concentration in marine and fresh waters.

This European Standard is comprised of the following:

- definition of the equipment requirement;
- *a priori* data and mathematical tools;
- recommendations for verification of measurement system performance and consideration of factors that can influence measurements;
- listing of the procedures to be implemented.

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.

ENV 13005, *Guide to the expression of uncertainty in measurement*

3 Terms and definitions

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

3.1 absorption coefficient

a

natural logarithm of the ratio between the light intensity entering and corresponding intensity emerging directly through a sample of water divided by the sample path length (in metres) in cases where the scattering of light is negligible

Note 1 to entry: The unit is m^{-1} .

Note 2 to entry: A spectrophotometer often gives the Log_{10} of the ratio in place of the natural logarithm.

3.2 extinction

c

sum of losses of directly transmitted light by absorption and scattering

Note 1 to entry: The unit is m^{-1} .

Note 2 to entry: The extinction *c* is related to absorption *a* and scattering *b*, by $c = a + b$.

3.3 extractive photometric EP

method of chlorophyll concentration estimation involving extraction and absorption measurement