SLOVENSKI STANDARD
SIST EN 17716:2025

01-februar-2025

Nadomešča:
SIST-TS CEN/TS 17716:2023

Rastlinski biostimulanti - Določanje Escherichia coli

Plant biostimulants - Determination of Escherichia coli

Pflanzen-Biostimulanzien - Bestimmung von Escherichia coli

Biostimulants des végétaux - Détermination des Escherichia coli

Ta slovenski standard je istoveten z: **EN 17716:2024**

ICS:

65.080	Gnojila	Fertilizers

SIST EN 17716:2025 **en,fr,de**

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

EN 17716

November 2024

ICS 65.080

Supersedes CEN/TS 17716:2022

English Version

Plant biostimulants - Determination of *Escherichia coli*

Biostimulants des végétaux - Détermination des *Escherichia coli*

Pflanzen-Biostimulanzien - Bestimmung von *Escherichia coli*

This European Standard was approved by CEN on 26 August 2024.

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, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Türkiye and United Kingdom.

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

CEN-CENELEC Management Centre: Rue de la Science 23, B-1040 Brussels

Ref. No. EN 17716:2024 E

Contents

Page

European foreword

This document (EN 17716:2024) has been prepared by Technical Committee CEN/TC 455 “Plant biostimulants”, the secretariat of which is held by AFNOR.

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 May 2025, and conflicting national standards shall be withdrawn at the latest by May 2025.

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

This document supersedes CEN/TS 17716:2022.

EN 17716:2024 includes the following significant technical changes with respect to CEN/TS 17716:2022:

— the Introduction has been updated and Table 1 has been removed;

— normative references have been updated;

— in Clause 3, new terms and definitions have been added and others have been revised;

— Annexes A, B and C have been revised.

— Annex ZA has been added.

This document has been prepared under a standardization request addressed to CEN by the European Commission. The Standing Committee of the EFTA States subsequently approves these requests for its Member States.

For the relationship with EU Legislation, see informative Annex ZA, which is an integral part of this document.

Any feedback and questions on this document should be directed to the users’ national standards body. A complete listing of these bodies can be found on the CEN website.

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, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Türkiye and the United Kingdom.

Introduction

The European Committee for Standardization (CEN) was requested by the European Commission (EC) to draft European Standards or European Standardization deliverables to support the implementation of Regulation (EU) 2019/1009 of 5 June 2019 [1] laying down rules on the making available on the market of EU fertilising products ("FPR" or "Fertilising Products Regulation").

This standardization request, presented as SR M/564 and relevant amendments, also contributes to the Communication on "Innovating for Sustainable Growth: A Bio economy for Europe". The interest in plant biostimulants has increased significantly in Europe as a valuable tool to use in agriculture. Standardization was identified as having an important role in order to promote the use of biostimulants. The work of CEN/TC 455 seeks to improve the reliability of the supply chain, thereby improving the confidence of farmers, industry, and consumers in biostimulants, and will promote and support commercialisation of the European biostimulant industry.

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 in accordance with this document be carried out by suitably trained staff.

1 Scope

This document gives guidance for the detection and identification of the specified microorganism *Escherichia coli* in technical and formulated plant biostimulants, both in liquid and solid states, and also the horizontal method for the enumeration of *ß*-glucuronidase-positive *E. coli* in plant biostimulants (both in liquid and solid states).

The qualitative method described in this document is based on the detection of *E. coli* in a non-selective liquid medium (enrichment broth), followed by isolation on a selective agar. Other methods can be appropriate, depending on the level of detection required.

NOTE 1 For the detection of *E. coli*, subcultures can be performed on non-selective culture media followed by suitable identification steps (e.g. using identification kits).

The quantitative method described in this document uses a colony-count technique at 44 °C ± 1 °C on a solid medium containing a chromogenic ingredient for detection of the enzyme *ß*-glucuronidase.

NOTE 2 Strains of *E. coli* which do not grow at 44 °C ± 1 °C and, in particular, those that are *ß*-glucuronidase negative, such as *E. coli* O157, will not be detected.

This document is applicable to the blends of fertilizing products where a blend is a mix of at least two of the following component EU fertilising products categories: Fertilizers, Liming Materials, Soil Improvers, Growing Media, Plant Biostimulants, and where the following category Plant Biostimulants is the highest % in the blend by mass or volume, or in the case of liquid form by dry mass. If Plant Biostimulants is not the highest % in the blend, the European Standard for the highest % of the blend applies. In case a blend of fertilizing products is composed of components in equal quantity or in case the component EU fertilising products used for the blend have identical formulations[1], the user decides which standard to apply.

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.

EN 17708:2024, *Plant biostimulants — Preparation of sample for microbial analysis*

EN 17714:2024, *Plant biostimulants — Determination of microrganims concentration*

EN 17702-1:2024, *Plant biostimulants — Sampling and sample preparation — Part 1: Sampling*

EN 17724:2024, *Plant biostimulants — Terminology*

EN ISO 21148:2017, *Cosmetics — Microbiology — General instructions for microbiological examination (ISO 21148:2017)*

[1] An example of such a blend is a product with 2 claimed functions consisting of a non-microbial plant biostimulant and an organic fertilizer composed of 1kg/kg of plant biostimulant from seaweed

3 Terms and definitions

For the purposes of this document, the terms and definitions given in EN 17724:2024 and the following apply.

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

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

3.1
Escherichia coli
Gram-negative rod, motile, smooth colonies, member of *Enterobacteriaceae*

Note 1 to entry: The main characteristics for identification are catalase positive, oxidase negative, fermentation of lactose, production of indole, growth on selective agar containing bile salts with characteristic colonies.

Note 2 to entry: *E. coli* can be isolated from moist environmental sources (air, water, soil) and is a faecal contamination indicator.

3.2
enrichment broth
non-selective liquid medium containing suitable neutralizers (according to Annex B) and/or dispersing agents and demonstrated to be suitable for the plant biostimulant under test

3.3
ß*-glucuronidase-positive *E. coli
bacteria which form typical blue to blue-green colony on Tryptone-Bile-Glucuronide agar (TBX) after incubation at 44 °C ± 1 °C for 24 hours

4 Principle

4.1 Qualitative method

The first step of the qualitative procedure is to perform an enrichment by using a non-selective broth medium to increase the number of microorganisms without the risk of inhibition by the selective ingredients that are present in selective/differential growth media.

The second step of the test (isolation) is performed on a selective medium followed by identification tests.

The presence or absence of *E. coli* per gram or per millilitre of sample is calculated (according to Clause 9).

4.2 Quantitative method

In the quantitative method, duplicate plates of TBX are inoculated with the specified quantity of the test sample (if the product is liquid) or the initial suspension.

Under the same conditions, using decimal dilutions of the test sample or of the initial suspension, two plates per dilution shall be inoculated.

The dishes are incubated for 18 h to 24 h at 44 °C ± 1 °C then examined to detect the presence of colonies which, from their characteristics, are considered to be *ß*-glucuronidase-positive *E. coli*.

The number of colony-forming units (CFU) of *ß*-glucuronidase-positive *E. coli* per gram or per millilitre of sample is calculated (according to Clause 9).

5 Diluent and culture media

5.1 General

The following diluents and culture media are suitable for the detection of *E. coli* and the enumeration of *ß*-glucuronidase-positive *E. coli* according to the proper procedure. Other diluents and culture media may be used if they have been demonstrated to be suitable for use.

Diluents and culture media should be prepared using the descriptions provided or from reagents/dehydrated culture media, according to the instructions from the manufacturer. The instructions provided by the supplier of the media/reagents should be followed for storage conditions, expiry date and use.

NOTE Ready-to-use diluents and media may be used when their composition and/or growth yields are comparable to those of the formulae given in the present document.

5.2 Broth and culture media in the qualitative method

5.2.1 Enrichment broth

The enrichment broth shall be used in the qualitative method to disperse the sample and to increase the initial microbial population. A non-exhaustive list and recipes of the possible enrichment broths are given in Annex A.

5.2.2 Selective culture media: Tryptone-Bile-Glucuronic agar (TBX) for isolation of *E. coli*

The selective agar shall be used in the qualitative method for the isolation and identification of *E. coli*. The list and recipes of the selective agar are given in Annex A.

5.3 Diluent and culture media in the quantitative method

5.3.1 Diluent

The list and recipes of the usable diluent in the preparation of the initial suspension and further decimal dilution is not part of the method specified in this document: EN 17708:2024 shall be used.

5.3.2 Culture media: Tryptone-Bile-Glucuronic agar (TBX)

The list and recipes of the culture medium that shall be used in the inoculation by plating technique of the initial suspension and further decimal dilutions are given in Annex A.

6 Apparatus and glassware

The laboratory equipment, apparatus and glassware typical of microbiological laboratory according to EN 17708:2024 shall be used.

7 Handling of plant biostimulants and sampling

The laboratory shall receive a sample which is truly representative and has not been damaged or changed during transport or storage.

Sampling is not part of the method specified in this document: EN 17702-1:2024 shall be used.

If necessary, the sample to be tested should be equilibrated at room temperature before starting the analysis.