SLOVENSKI STANDARD
SIST EN 17720:2025

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Nadomešča:
SIST-TS CEN/TS 17720:2023

Rastlinski biostimulanti - Določanje enterokokov

Plant biostimulants - Determination of enterococci

Pflanzen-Biostimulanzien - Bestimmung von enterococci

Biostimulants des végétaux - Détermination des enterococci

Ta slovenski standard je istoveten z: **EN 17720:2024**

ICS:

65.080	Gnojila	Fertilizers

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English Version

Plant biostimulants - Determination of enterococci

Biostimulants des végétaux - Détermination des enterococci

Pflanzen-Biostimulanzien - Bestimmung von enterococci

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

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

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European foreword

This document (EN 17720:2024) 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of an identical text or by endorsement, at the latest by May 2025, and conflicting national standards shall be withdrawn at the latest by May 2025.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 17720:2022.

EN 17720:2024 includes the following significant technical changes with respect to CEN/TS 17720:2022:

— the title;

— the European foreword and Introduction have been updated for consistency with other standards;

— the Scope has been reworded for clarification;

— the normative references have been updated;

— in Clause 3 the definitions of presumptive enterococci and enterococci have been revised;

— in Clause 7 and Clause 8 the sampling and sample preparation instructions have been replaced by the relevant normative references;

— in Clause 9, the procedure has been clarified and references to the media and apparatus required have been added.

— Annex A and Annex B have been revised for consistency with other standards.

— Annex ZA has been added in line with the clarified Scope of the method.

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 relationship with EU Directive(s) / Regulation(s), 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 specifies a method for the enumeration of enterococci in microbial and non-microbial plant biostimulants. This document specifies a colony-count technique on a selective medium (Slanetz-Bartley agar) with confirmation on Bile Esculin Azide agar.

This document is applicable to all formulations of microbial and non-microbial plant biostimulants in liquid or solid form. This document is not applicable to other fertilizing products.

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 ISO 7218:2024, *Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations*

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

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

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

EN 17724:2024, *Plant biostimulants — Terminology*

[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
presumptive enterococci
Gram-positive, catalase-negative cocci, able to reduce 2,3,5-triphenyl tetrazolium chloride to formazan on Slanetz-Bartley agar under the conditions specified in this document

3.2
enterococci
bacteria which are able to reduce 2,3,5-triphenyltetrazolium chloride to formazan on the surface of a selective culture medium containing sodium azide (Slanetz-Bartley agar) and to hydrolyse esculin at 44 °C on a medium containing bile salts (Bile Esculin Azide agar), resulting in blackening of the medium under the conditions specified in this document

4 Principle

From the initial suspension, decimal dilutions (serial ten-fold dilutions) shall be prepared immediately before use to prevent the suspension from settling. Each dilution shall be shaken immediately before 0,1 ml from each dilution are plated onto Slanetz-Bartley agar. This selective medium (as described in EN ISO 7899-2:2000 [2]) inhibits the growth of Gram-negative bacteria and *Staphylococcus.* Plates are incubated at 36 °C ± 2 °C for 44 h under aerobic conditions. Presumptive enterococci colonies show a pink-red or red-brown colour on Slanetz-Bartley agar.

If reaching a lower detection limit is required, 1 ml of the undiluted biostimulant product or 1 ml of the first dilution (10^{-1}) can be spread over the surface of Slanetz-Bartley agar plates (140 mm of diameter or 3 smaller plates having a diameter of 90 mm).

Presumptive enterococci colonies are counted and the number of colony-forming units (CFU) per g or ml is calculated. Presumptive enterococci shall be further confirmed by subsequent inoculation onto BEA agar pre-heated plates. The pre-heating of the BEA agar plates is carried out for 2 h at 44 °C ± 1 °C. BEA agar plates are inoculated with presumptive colonies from Slanetz-Bartley agar and incubated aerobically for 24 h ± 2 h at 44 °C ± 1 °C.

To verify the colony count, a phenotypic characterization and confirmation of a selection of colonies can be done by means of an identification kit (widely available on the market).

5 Diluents, selective media and test kit for phenotypic characterization

5.1 General

For current laboratory practice, EN ISO 7218:2024 shall be followed. Performance testing of culture media is recommended to be done in accordance with standards comparable to EN ISO 11133:2014 [3].

Composition of culture media and reagents and their preparation are described in Annex A (normative).

5.2 Selective media

Slanetz-Bartley agar and BEA agar (commercially available from various suppliers) are used as selective media. These selective media shall be prepared in accordance with Annex A.

5.3 Phenotypic characterization and confirmation

To avoid false-positives, a catalase test can be carried out on doubtful colonies. Enterococci exhibit a negative reaction to the catalase test. If positive, consider the colony as non-enterococci.

A microscopic examination can also be performed, by wet-mount at (x1 000) magnification. Verify up to 5 colonies.

NOTE Microscopic examination of selected colonies typically shows diplococci or short chains of cocci.

6 Apparatus and glassware

Usual microbiological laboratory equipment and, in particular, the following.

6.1 Equipment for dry sterilization (oven) or wet sterilization (autoclave), the specifications in EN ISO 7218:2024 shall be followed.

6.2 Incubator, capable of maintaining a temperature of 44 °C ± 1 °C and 36 °C ± 2 °C.

6.3 Water bath, capable of maintaining a temperature of 47 °C ± 2 °C.

6.4 Mechanical mixer/shaker, Vortex Mixer or equivalent.

6.5 Magnetic stirrer.

6.6 Balance, capable of weighing to two decimal places.

6.7 Screw-cap tubes, bottles, or flasks of appropriate capacity (25 ml bottles, test tubes and flasks; 1 000 ml Duran bottles).

6.8 Pipette and sterile tips of appropriate volume.

6.9 Sterile Petri dishes, 90 mm or 140 mm in diameter.

6.10 Microscope, magnification 1 000x.

6.11 Sterile membrane filters, of 0,2 µm.

6.12 pH meter, with an accuracy of ± 0,1 pH unit at 20 °C to 25 °C.

6.13 Cooling unit, operating at 5 °C ± 3 °C.

7 Sampling

A representative sample of 25 g or 25 ml of the plant biostimulant shall be obtained according to EN 17702-1:2024.

8 Preparation of test sample

The sample obtained shall be suspended on phosphate-buffered saline (PBS) to prepare an initial suspension according to the protocol for enumeration of non-beneficial microorganisms specified in EN 17708:2024. Decimal dilutions shall be prepared from the initial suspension according to the procedure for further dilutions specified in EN 17708:2024.

9 Procedure

9.1 Inoculation and incubation on Slanetz-Bartley agar

An initial suspension and decimal dilutions shall be prepared according to the instructions provided in this document (8). Pour plate and spread plate techniques may be used to determine the presence of enterococci in the sample.

For pouring plates: using a sterile pipette (6.8), transfer 1 ml of the initial suspension and decimal dilutions to a sterile Petri dish (6.9). Pour, into each Petri dish, approximately 15 ml of Slanetz-Bartley agar, previously cooled at 47 °C ± 2 °C inside a water bath (6.3). Carefully mix the inoculum with the medium and allow the mixture to solidify.

The time between the distribution of the inoculum in a dish and the pouring of the medium shall not exceed 15 min.

For spreading plates: separately transfer 0,1 ml or 1 ml of the initial suspension and decimal dilutions onto two Petri dishes with Slanetz-Bartley agar each.

NOTE 1 ml can be spread in one 140 mm petri dish or in three 90 mm petri dishes (6.9).

All Slanetz-Bartley agar plates shall be incubated aerobically at 36°C ± 2 °C for 44 h ± 4 h (6.2).

9.2 Evaluation of results

After the specified period of incubation, the typical colonies in each plate (9.1) containing less than 150 typical CFU and less than 300 total (typical and non-typical) CFU shall be counted. All raised colonies which show a red, brown or pink colour, either in the centre or throughout the colony, shall be considered as typical.

NOTE 1 Colony description:

— circular;

— convex to dome-shaped;

— entire;

— glistening surface;

— pink, red, brown colour;

— opaque.

NOTE 2 Colony size varies between 0,5 mm and 2 mm in diameter.

If there are typical colonies (presumptive enterococci), proceed to the confirmation step on BEA agar (9.3).

9.3 Confirmation step on BEA

From the plates obtained in 9.2, select typical colonies to confirm (up to 5 colonies).