SLOVENSKI STANDARD
SIST EN 17719:2025

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Nadomešča:
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Rastlinski biostimulanti - Ugotavljanje števila anaerobnih mikroorganizmov na mikrotitrskih ploščah

Plant biostimulants - Determination of the anaerobic plate count

Pflanzen-Biostimulanzien - Bestimmung der anaeroben Keimzahl

Biostimulants des végétaux - Détermination du dénombrement sur plaque des germes anaérobies

Ta slovenski standard je istoveten z: **EN 17719:2024**

ICS:

65.080	Gnojila	Fertilizers

SIST EN 17719:2025 **en,fr,de**

EUROPEAN STANDARD
NORME EUROPÉENNE
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EN 17719

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ICS 65.080

Supersedes CEN/TS 17719:2022

English Version

Plant biostimulants - Determination of the anaerobic plate count

Biostimulants des végétaux - Détermination du dénombrement sur plaque des germes anaérobies

Pflanzen-Biostimulanzien - Bestimmung der anaeroben Keimzahl

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

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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.

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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 17719:2024) 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of an identical text or by endorsement, at the latest by May 2025, and conflicting national standards shall be withdrawn at the latest by May 2025.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 17719:2022.

EN 17719:2024 includes the following significant technical changes with respect to CEN/TS 17719:2022:

— The European foreword and the introduction have been updated;

— The Normative references reported in Clause 2 have been updated;

— The definitions have been revised;

— Clauses 7, 8, and 10 have been revised;

— Annexes A, and B have been revised;

— Annex C on the results of the interlaboratory study for the determination of the anaerobic plate count has been added;

— 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 specifies a horizontal method for the enumeration of microorganisms that are able to grow and form colonies in a solid medium after anaerobic incubation at 30 °C.

The method applies to microbial plant biostimulants, except those composed of aerobic bacteria.

This method does not apply to the microbiological monitoring of the environment in which microbial plant biostimulants are manufactured.

No information about potential human pathogens can be inferred from anaerobic plate counts.

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 percentage in the blend by mass or volume, or in the case of liquid form by dry mass. If Plant Biostimulants is not the highest percentage in the blend, the European Standard for the highest percentage 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) shall apply.

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

EN 17724:2024, *Plant biostimulants — Terminology*

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

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

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

EN ISO 11133:2014[2], *Microbiology of food, animal feed and water — Preparation, production, storage and performance testing of culture media (ISO 11133:2014)*

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

[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 1 kg/kg of plant biostimulant from seaweed."

[2] As amended by EN ISO 11133:2014/A1:2018 and EN ISO 11133:2014/A2:2020.

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

3.1
microorganism
any microbiological entity, including lower fungi, bacteria, and viruses, cellular or non-cellular, capable of replication or of transferring genetic material

[SOURCE: Regulation (EC) No 1107/2009, Article 3, point 15]

3.2
anaerobic bacteria
any microorganism capable of both aerobic and anaerobic metabolism or that only lives and grows in the absence of molecular oxygen

[SOURCE: EN ISO 11139:2018, 3.114 modified]

[SOURCE: EN ISO 11139:2018, 3.186 modified]

3.3
plate count
value established from the enumeration of recoverable colony-forming units (CFU)

[SOURCE: EN ISO 11139:2018, 3.316 modified]

4 Principle

4.1 General

Viable anaerobic bacteria shall be enumerated by the plate count technique under an anaerobic atmosphere [2]. Caution shall be exercised when applying the method since isolates may be pathogenic.

4.2 Brief description

A fixed amount of a dilution of the test sample shall be placed in an empty Petri dish and mixed in a specified melted agar culture medium to create a pour plate.

The plates shall be incubated at 30 °C ± 1 °C under anaerobic conditions for 48 h to 72 h.

The number of microorganisms per gram or millilitre of test sample shall be calculated as specified in Clause 9.

5 Culture media and reagents

For current laboratory practice, refer to EN 17708:2024 and EN ISO 11133:2014[2].

The composition of Anaerobe Agar (ANA) [3] and reagents, as well as their preparation, are described in Annex B.

6 Equipment and consumables

6.1 Special apparatus, anaerobic incubator, BBL GasPak or equivalent, equipped with GasPak hydrogen and CO_2 generator envelopes with an anaerobic indicator.

6.2 Apparatus for dry sterilization (oven) or wet sterilization (autoclave), according to EN ISO 7218:2024.

6.3 Drying cabinet or incubator, capable of operating at 30 °C ± 2 °C.

6.4 Water bath, capable of operating at 47 °C ± 2°C.

6.5 pH-meter, having a maximum permissible error of ± 0,1 pH unit at 25 °C.

6.6 Sterile graduated pipettes or automatic pipettes of nominal capacities of 0,1 ml, 0,5 ml, 1 ml, and 10 ml.

6.7 Sterile Petri dishes, with a diameter of approximately 90 mm and (optional) large size (diameter of approximately 140 mm).

6.8 Refrigerator, capable of operating at 5 °C ± 3 °C.

6.9 Peristaltic blender (Stomacher®[3]) with 400 ml sterile bags.

6.10 Blender motor and jars or vortex.

7 Preparation of test sample

7.1 General

A representative sample of the product shall be obtained as specified in EN 17702-1:2024 and the sample shall be prepared according to the following procedure. To prepare the analytical unit, liquids or free-flowing materials shall be agitated until the contents are homogeneous. If the sample is solid, the analytical unit shall be obtained by taking a portion from several locations within the sample. To reduce the workload, the analytical units may be combined for analysis. It is recommended that a composite contain no more than five analytical units.

General rules for the preparation of the initial suspension for microbiological examination are specified in EN 17708:2024

A representative sample of the product shall be prepared, considering the different formulations of plant biostimulants. To minimize the effect of sampling on the significance and the reliability of the analytical results, the number of replicates tested shall be increased to 5.

7.2 Liquid (water-based) formulations

A portion of 25 ml of the product (the analytical unit) shall be aseptically added to a 400 ml sterile bag (6.9) or in a blender jar (6.10) containing 225 ml of an appropriate sterile diluent (e.g. Buffered Peptone Water) maintained at room temperature. The sample shall be blended, stomached, or vortexed as required for thorough mixing.

7.3 Liquid (oil-based) emulsifiable concentrate (EC) formulations

A portion of 25 ml of the product (the analytical unit) shall be aseptically added to a 400 ml sterile bag (6.9) or in a blender jar (6.10) containing 225 ml of an appropriate sterile diluent (e.g. Buffered Peptone Water) maintained at room temperature. The sample shall be blended, stomached, or vortexed as required for thorough mixing.

[3] Stomacher® is 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 CEN of this product.

7.4 Solid wettable powder (WP) formulations

A portion of 25 g of the product (the analytical unit) shall be aseptically added to a 400 ml sterile bag (6.9) containing 225 ml of an appropriate sterile diluent (e.g. Buffered Peptone Water) maintained at room temperature. The mixture shall be homogenized for 2 min at the highest speed with a peristaltic blender (6.9).

7.5 Solid water dispersible granules (WDG) formulations

A portion of 25 g of the product (the analytical unit) shall be aseptically added to a 400 ml sterile bag (6.9) containing 225 ml of an appropriate sterile diluent (e.g. Buffered Peptone Water) maintained at room temperature. The mixture shall be homogenized for 2 min at the highest speed with a peristaltic blender (6.9).

7.6 Solid pellets, granules, microgranules (slow release) formulations

A portion of 25 g of the product (the analytical unit) shall be aseptically added to a 400 ml sterile bag (6.9) containing 225 ml of an appropriate sterile diluent (e.g. Buffered Peptone Water) maintained at room temperature. The mixture shall be homogenized for 2 min at the highest speed with a peristaltic blender (6.9).

7.7 Solid substrates

A portion of 25 g of the product (the analytical unit) shall be aseptically added to a 400 ml sterile bag (6.9) containing 225 ml of an appropriate sterile diluent (e.g. Buffered Peptone Water) maintained at room temperature. The mixture shall be homogenized for 2 min at the highest speed with a peristaltic blender (6.9).

8 Procedure

8.1 Test portion, initial suspension, and dilutions

For the preparation of the initial suspension, an appropriate sterile diluent (e.g. Buffered Peptone Water, described in B.1.4) shall be used. A portion of 25 g or 25 ml of the sample shall be aseptically weighed and put into 225 ml of the diluent to yield a tenfold dilution (refer to EN 17708:2024. A volume of 1 ml ± 0,02 ml of the primary dilution shall be aseptically transferred into 9 ml ± 0,02 ml of the diluent, obtaining the dilution 10^{-2}. Further ten-fold serial dilutions shall be prepared (adding 1 ml ± 0,02 ml of the previously diluted sample into 9 ml ± 0,02 ml of the diluent) according to the expected level of contamination of the product, e.g. until reaching the dilution 10^{-5}.

8.2 Inoculation and incubation

A volume of 1 ml of each sample dilution shall be pipetted into a Petri dish. If the laboratory operates under the quality assurance ISO/IEC 17025:2017 [5], one plate per dilution can be used if at least two successive 10-fold dilutions are carried out. In the case of liquid biostimulant products, 1 ml of the undiluted product and of the two successive 10-fold dilutions shall be inoculated. Approximately 15 ml of ANA agar cooled to 47 °C ± 2 °C shall be poured into each dish. Plates shall be swirled and the agar medium shall be allowed to solidify. Immediately after solidification the plates shall be inverted and placed in an anaerobe jar. The anaerobic atmosphere shall be generated following the manufacturer's instructions.

The anaerobe jar shall be incubated at 30 °C ± 2 °C (6.3) for 48 h to 72 h.

For CFU counting on plates, EN 17714:2024 shall be referred to.

Appropriate negative controls (diluent-only) shall be run concurrently with the sample serial dilutions.