

SLOVENSKI STANDARD SIST EN 17717:2025

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Rastlinski biostimulanti - Ugotavljanje prisotnosti salmonele (Salmonella spp.)

Plant biostimulants - Detection of Salmonella spp.

Pflanzen-Biostimulanzien - Nachweis von Salmonella spp.

Biostimulants des végétaux - Recherche des Salmonella spp.

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Ta slovenski standard je istoveten z: EN 17717:2024

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English Version

Plant biostimulants - Detection of Salmonella spp.

Biostimulants des végétaux - Recherche des Salmonella spp. Pflanzen-Biostimulanzien - Nachweis von Salmonella spp.

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.

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

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

EN 17717:2024 (E)

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EN 17717:2024 (E)

European foreword

This document (EN 17717:2024) 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of an identical text or by endorsement, at the latest by May 2025, and conflicting national standards shall be withdrawn at the latest by May 2025.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 17717:2022.

EN 17717:2024 includes the following significant technical changes with respect to CEN/TS 17717:2022:

- the European foreword and introduction have been updated;
- in Table 1, errors in the interpretation of the serological reactions have been corrected;
- in Annex B, the layout of Table B.1 has been improved;
- in Annex C, the footnotes have been revised; tandards
- Annex D (method validation study) has been added;
- Annex ZA has been added. **Document Preview**

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.

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1 Scope

This document specifies a method for the detection of *Salmonella* spp. in biostimulants.

This document is applicable to all microbial biostimulants in agriculture.

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 formulations¹, 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 17724:2024, Plant Biostimulants — Terminology

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

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

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

3.1

Salmonella spp.

microorganisms which form typical colonies on solid selective media and which display the morphological, physiological and biochemical characteristics described when the analysis is carried out in accordance with this document

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 amonded by FN ISO 11132:2014 (A1:2019 and FN ISO 11132:2014 (A2:2020

 $^{^{\}rm 2}$ As amended by EN ISO 11133:2014/A1:2018 and EN ISO 11133:2014/A2:2020

3.2

detection of Salmonella spp.

determination of the detection or not detection of *Salmonella* spp. (3.1), in 25 g or 25 ml of product, when tests are carried out in accordance with this document

3.3

laboratory sample

sample intended for laboratory inspection or testing

3.4

test sample

sample prepared from the laboratory sample and from which test portions will be taken

3.5

test portion

quantity of material taken from the test sample (or if both are the same, from the laboratory sample) and on which the test or observation is carried out

4 Principle

4.1 General

The detection of *Salmonella* spp. requires three successive steps as specified in Annex A. The three steps are the selective enrichment, the isolation on a chromogenic agar, and the confirmation with a serological test (and if required, a selective media).

NOTE *Salmonella* spp. can be present in small numbers and are often accompanied by considerably larger numbers of other bacteria, such as *Enterobacteriaceae* or of other families. Enrichment is used to allow the detection of low numbers of *Salmonella* spp. or stressed *Salmonella* spp.

Stressed microorganisms are defined here as those present in the environment that can be injured or that can have developed in harsh environments. Such organisms can be difficult to detect because they struggle to grow on selective media. However, under suitable conditions, they can repair the cellular damages and recover their normal properties.

4.2 Enrichment in selective liquid medium

Buffered Peptone Water (BPW) containing 10 mg/l Novobiocin at room temperature is inoculated with the test portion, then incubated from 34 °C to 38 °C for 18 h \pm 2 h.

For large quantities (e.g. 1 l or more), pre-warm the BPW to between 34 °C and 38 °C before mixing it with the test portion.

4.3 Plating out on selective solid media

From the enrichment obtained in 4.2, the chromogenic solid media (5.2) is inoculated.

This selective agar is incubated from $34 \degree C$ to $38 \degree C$ for $24 h \pm 3 h$ (or according to the manufacturer's instructions if explicitly recommended).

4.4 Confirmation

Colonies of presumptive *Salmonella* spp. are confirmed by means of appropriate serological testing. If the serological test gives a negative result, the inoculation of a selective agar (B.5) is required.

If the test gives a negative result, up to 4 other presumptive colonies will be tested (if possible and up to 5 colonies in total).

5 Culture media, reagents, antisera

5.1 General

For current laboratory practice, EN ISO 7218:2024 and EN ISO 11133:2014² shall be used.

Composition of culture media and reagents and their preparation are described in Annex B.

5.2 Isolation chromogenic agar

This isolation medium is chosen by the testing laboratory and shall highlight the C8-esterase enzymatic activity. For examples of isolation media, see Annex C, Table C.1.

5.3 Non-selective agar

General purpose agar supporting the growth of a wide range of non-fastidious strains. See B.4.

5.4 Confirmation selective agar

This isolation medium is chosen by the testing laboratory and shall highlight the production of hydrogen sulphide (H_2S) by the strains (see B.5). For examples of isolation media, see Annex C, Table C.2.

6 Equipment and consumables

Disposable equipment is an acceptable alternative to reusable glassware if it has suitable specifications.

Usual microbiological laboratory equipment as described in EN ISO 7218:2024 shall be used and, in particular, the following.

6.1 Apparatus for dry sterilization (oven) or wet sterilization (autoclave)

6.2 Drying cabinet or oven, capable of operating between 25 °C and 50 °C.

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6.3 Incubator(s), capable of operating from 34 °C to 38 °C and at 37 °C ± 1 °C.

6.4 Water bath, capable of operating from 47 °C to 50 °C.

6.5 Water bath, capable of operating at $37 \degree C \pm 1 \degree C$.

It is recommended to use a water bath (6.4 and 6.5) containing an antibacterial agent because of the low infective dose of *Salmonella* spp.

6.6 Cooling unit, adjustable at 5 °C ± 3 °C.

6.7 Freezer, capable of operating at -20 °C ± 5 °C.

6.8 Sterile loops with a diameter of approximately 3 mm (10 μl volume).

6.9 pH-meter having an accuracy of calibration of ± 0,1 pH unit from 20 °C to 25 °C.

6.10 Sterile tubes, bottles, or flasks with caps, of appropriate capacity.

6.11 Sterile graduated pipettes or automatic pipettes of nominal capacities of 25 ml, 10 ml, 1 ml, and 0,1 ml.

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